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Research Article

Morphological and phylogenetic analyses reveal eight novel species of *Pestalotiopsis* (Sporocadaceae, Amphisphaeriales) from southern China

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Abstract

Plants play an important role in maintaining the ecological balance of the biosphere, but often suffer from pathogenic fungi during growth. During our continuing mycological surveys of plant pathogens from terrestrial plants in Jiangxi and Yunnan provinces, China, 24 strains of *Pestalotiopsis* isolated from diseased and healthy tissues of plant leaves represented eight new species, viz. *P. alpinicola*, *P. camelliicola*, *P. cyclosora*, *P. eriobotryae*, *P. gardeniae*, *P. hederae*, *P. machiliana* and *P. mangifericola*. Multi-locus (ITS, *tef1-α* and *tub2*) phylogenetic analyses were performed using maximum-likelihood and Bayesian inference to reveal their taxonomic placement within *Pestalotiopsis*. Both molecular phylogenetic analyses and morphological comparisons supported them as eight independent taxa within *Pestalotiopsis*. Illustrations and descriptions of these eight taxa were provided, in conjunction with comparisons with closely related taxa in the genus. This work highlights the large potential for new fungal species associated with diseased plant leaves.

Key words: Asexual Ascomycota, molecular phylogeny, new species, Sordariomycetes, taxonomy

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Introduction

Fungi are widely distributed and highly diverse in nature, forming large and complex ecosystems that play crucial roles in many biological processes (Schimann et al. 2017). Current estimates of fungal diversity are highly uncertain, ranging from 1.5 to 12 million species (Wu et al. 2019; Hyde et al. 2021; Bhunjun et al. 2022). The abundance of fungi remains to be unexplored, and only 10% of fungi were currently described (Hyde et al. 2021), but most species lack the molecular data before the advent of Sanger sequencing. In recent years, with the development of molecular techniques, the DNA-based species delimitation techniques are maturing gradually and have become an important approach to evaluate the fungal phylogenetic relationships and taxonomic placements in the study of modern fungal classification.

Pestalotiopsis Steyaert is a species-rich asexual genus with conidial appendages in the family Sporocadaceae Corda (Barr 1975, 1990; Kang et al. 1998, 1999), which was originally introduced to accommodate those *Pestalotia*-like species that have 5-celled conidia rather than 6-celled conidia (Steyaert 1949), and such a morphological distinction was subsequently supported by further evidence of the electronic microscopy (Guba 1961; Steyaert 1963; Griffiths and Swart 1974a,b; Sutton 1980). For *Pestalotiopsis* species, the traditional taxonomy of delineating interspecific relationships is mainly based on morphological characteristics, and most species are distinguished by conidial dimensions (Maharachchikumbura et al. 2011). Based on morphological and multi-locus phylogenetic analyses, Maharachchikumbura et al. (2014) proposed two segregated anamorphic genera from *Pestalotiopsis*, namely *Neopestalotiopsis* Maharachch., K.D. Hyde & Crous and *Pseudopestalotiopsis* Maharachch., K.D. Hyde & Crous to accommodate *Pestalotiopsis* species. *Neopestalotiopsis* is distinguished from *Pestalotiopsis* and *Pseudopestalotiopsis* by its multicolored median cells, and *Pseudopestalotiopsis* has three darker median cells compared to *Pestalotiopsis*.

To data, about 437 epithets for *Pestalotiopsis* have been listed in Index Fungorum (Index Fungorum 2024). Members of the genus are widely distributed in tropical and temperate regions as endophytes, plant pathogens or saprobes (Bate-Smith and Metcalfe 1957; Maharachchikumbura et al. 2012, 2014), but occasionally, some species of *Pestalotiopsis* have been reported as mycoparasites, human and insect pathogens (Lv et al. 2011; Monden et al. 2013; Xie et al. 2014; Li et al. 2017). The genus *Pestalotiopsis* has received considerable attention in recent years, and more research on its species diversity is still needed.

China is considered an important Asian reservoir of biodiversity by the Convention on Biological Diversity. Its rich vegetation and varied climatic regimes create a very wide range of habitats favoring the development of various microbial species. During ongoing mycological surveys of plant pathogens from terrestrial plants in Jiangxi and Yunnan provinces, 24 *Pestalotiopsis* strains isolated from diseased plant leaves are obtained. Based on morphological and multi-locus (ITS, *tef1-α* and *tub2*) phylogenetic analyses, eight *Pestalotiopsis* species were proposed as new to science in the present study.

Materials and methods

Sample collection, fungal isolation and morphological characterization

Samples of plant disease leaves were collected from different habitats in Yunnan and Jiangxi provinces, China, labeled and returned to the laboratory in Ziploc™ bags. The tissue isolation method was used for the isolation and identification of pathogenic fungi in this study (Gao et al. 2014). The fresh leaves were washed with running water to remove dirt and dust, then tissue pieces of junction from the diseased and healthy parts of plant leaves were cut into small pieces (5 × 5 mm). The tissue pieces were surface-sterilized with 75% ethanol for 1 min and 5% sodium hypochlorite (NaClO) for 45 s, then washed

3 times with sterile distilled water for 20 s each time, placed on sterilized filter paper to dry out the water, the tissue pieces were transferred to the potato dextrose agar (PDA, 200 g potato, 20 g glucose, 20 g agar, and 1000 mL water) plates and incubated at 25 °C in darkness until spores germinated, and the hyphal tip of individual colonies were transferred to fresh PDA plates to obtain a pure culture for further study. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. Cultural characteristics were observed and recorded after 7 days. Morphological characteristics were examined using an Olympus BX 53 compound microscope and photographed using the Olympus DP 27 digital camera (Olympus Optical Co., Tokyo, Japan) with a 60 × objective at the same background color and scale, and the conidia were randomly selected for measurement. The studied specimens and cultures were deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, China (HJAUP). The names of the new taxa were registered in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp).

DNA extraction, PCR amplification and sequencing

When the single colonies on PDA were grown for 7 days, approximately 500 mg of fresh fungal mycelia were scraped for the total genomic DNA extraction using the Solarbio Fungi Genomic DNA Extraction Kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) following the manufacturer's protocol. To confirm the species, the regions (ITS, *tef1-α* and *tub2*) of all fungal isolates were sequenced. A portion of the internal transcribed spacer (ITS), translation elongation factor 1- alpha gene (*tef1-α*) and β-tubulin (*tub2*) loci were amplified using primers pairs ITS5/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999) and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The corresponding primer pairs and PCR processes are listed in Table 1. The PCR mixture consisted of 10 μL Power Taq PCR Master Mix, 7.4 μL double-distilled water (ddH$_2$O), 0.8 μL of each primer, and 1 μL template DNA were made up to the final volume of 20 μL. The PCR amplification products were checked via electrophoresis in 1% agarose gels and stained with ethidium bromide. Purification and sequencing of PCR products were carried out at Beijing Tsingke Biotechnology Co., Ltd., Beijing, China. The newly obtained sequences were deposited in NCBI GenBank (www.ncbi.nlm.nih.gov, accessed on 28 June Table 2).

Table 1. Primers and PCR program used in this study.

Locus	Primers		PCR Program
	Name	Sequence 5'–3'	
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	94 °C: 3 min, (94 °C: 15 s, 54 °C: 15 s, 72 °C: 30 s) ×35 cycles, 72 °C: 5 min
	ITS4	TCCTCCGCTTATTGATATGC	
tef1-α	EF1-728F	CATCGAGAAGTTCGAGAAGG	94 °C: 3 min, (94 °C: 15 s, 59.5 °C: 15 s, 72 °C: 30 s) ×35 cycles, 72 °C: 5 min
	EF1-986R	TACTTGAAGGAACCCTTACC	
tub2	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	94 °C: 3 min, (94 °C: 15 s, 55 °C: 15 s, 72 °C: 30 s) ×35 cycles, 72 °C: 5 min
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	

Table 2. Taxa used in the phylogenetic analyses and their GenBank accession numbers. New sequences are in bold.

Species	Strain Number	Host/Substrate	Locality	GenBank Accession Number		
				ITS	*tef1-a*	*tub2*
Pestalotiopsis abietis	CFCC 53011 T	*Abies fargesii*	China	MK397013	MK622277	MK622280
P. abietis	CFCC 53012	*Abies fargesii*	China	MK397014	MK622278	MK622281
P. adusta	ICMP 6088 T	Refrigerator door	Fiji	JX399006	JX399070	JX399037
P. adusta	MFLUCC 10–146	*Syzygium* sp.	Thailand	JX399007	JX399071	JX399038
P. aggestorum	LC 6301 T	*Camellia sinensis*	China	KX895015	KX895234	KX895348
P. aggestorum	LC 8186	*Camellia sinensis*	China	KY464140	KY464150	KY464160
P. alloschemones	CGMCC 3.23480 T	*Alloschemone occidentalis*	China	OR247981	OR361456	OR381056
P. alloschemones	LC15841	*Alloschemone occidentalis*	China	OR247982	OR361457	OR381057
P. alpinicola	**HJAUP C1644.221 T**	***Alpinia zerumbet***	**China**	**PP962274**	**PP952249**	**PP952219**
P. alpinicola	**HJAUP C1644.222**	***Alpinia zerumbet***	**China**	**PP962275**	**PP952248**	**PP952220**
P. anacardiacearum	IFRDCC 2397 T	*Mangifera indica*	China	KC247154	KC247156	KC247155
P. anhuiensis	CFCC 54791 T	*Cyclobalanopsis glauca*	China	ON007028	ON005045	ON005056
P. aporosae-dioicae	SAUCC224004 T	*Aporosa dioica*	China	OR733506	OR912988	OR912985
P. aporosae-dioicae	SAUCC224005	*Aporosa dioica*	China	OR733505	OR912989	OR912986
P. appendiculata	CGMCC 3.23550 T	*Rhododendron decorum*	China	OP082431	OP185509	OP185516
P. arceuthobii	CBS 434.65 T	*Arceuthobium campylopodum*	USA	KM199341	KM199516	KM199427
P. arengae	CBS 331.92 T	*Arenga undulatifolia*	Singapore	KM199340	KM199515	KM199426
P. australasiae	CBS 114126 T	*Knightia* sp.	New Zealand	KM199297	KM199499	KM199409
P. australasiae	CBS 114141	*Protea* sp.	New South Wales	KM199298	KM199501	KM199410
P. australis	CBS 111503	*Protea neriifolia × susannae* cv. 'Pink Ice'	South Africa	KM199331	KM199557	KM199382
P. australis	CBS 114193 T	*Grevillea* sp.	New South Wales	KM199332	KM199475	KM199383
P. biappendiculata	CGMCC 3.23487 T	*Rhododendron* sp.	China	OR247984	OR361459	OR381059
P. biappendiculata	LC4282	*Rhododendron* sp.	China	OR247990	OR361465	OR381065
P. biappendiculata	LC4283	*Rhododendron* sp.	China	OR247991	OR361466	OR381066
P. biciliata	CBS 124463 T	*Platanus × hispanica*	Slovakia	KM199308	KM199505	KM199399
P. biciliata	CBS 236.38	*Paeonia* sp.	Italy	KM199309	KM199506	KM199401
P. brachiata	LC 2988 T	*Camellia* sp.	China	KX894933	KX895150	KX895265
P. brachiata	LC 8188	*Camellia* sp.	China	KY464142	KY464152	KY464162
P. brachiata	LC 8189	*Camellia* sp.	China	KY464143	KY464153	KY464163
P. brassicae	CBS 170.26 T	*Brassica napus*	New Zealand	KM199379	KM199558	–
P. camelliicola	**HJAUP C1804.221 T**	***Camellia japonica***	**China**	**PP962357**	**PP952236**	**PP952229**
P. camelliicola	**HJAUP C1804.222**	***Camellia japonica***	**China**	**PP962358**	**PP952235**	**PP952230**
P. camelliae	MFLUCC 12–0277 T	*Camellia japonica*	China	JX399010	JX399074	JX399041
P. camelliae–oleiferae	CSUFTCC 08 T	*Camelliae oleiferae*	China	OK493593	OK507963	OK562368
P. camelliae–oleiferae	CSUFTCC 09	*Camelliae oleiferae*	China	OK493594	OK507964	OK562369
P. cangshanensis	CGMCC 3.23544 T	*Rhododendron delavayi*	China	OP082426	OP185510	OP185517
P. castanopsidis	CFCC 54430 T	*Castanopsis lamontii*	China	OK339732	OK358493	OK358508
P. castanopsidis	CFCC 54305	*Castanopsis hystrix*	China	OK339733	OK358494	OK358509
P. castanopsidis	CFCC 54384	*Castanopsis hystrix*	China	OK339734	OK358495	OK358510
P. chamaeropis	CBS 186.71 T	*Chamaerops humilis*	Italy	KM199326	KM199473	KM199391
P. chamaeropis	CFCC 55122	*Quercus aliena*	China	OM746229	OM840001	OM839902
P. chamaeropis	CFCC 55023	*Castanopsis fissa*	China	OM746233	OM840005	OM839906
P. changjiangensis	CFCC 54314 T	*Castanopsis tonkinensis*	China	OK339739	OK358500	OK358515
P. changjiangensis	CFCC 54433	*Castanopsis hainanensis*	China	OK339740	OK358501	OK358516

Species	Strain Number	Host/Substrate	Locality	GenBank Accession Number		
				ITS	*tef1-a*	*tub2*
P. changjiangensis	CFCC 52803	*Cyclobalanopsis austrocochinchinensis*	China	OK339741	OK358502	OK358517
P. chaoyangensis	CFCC 55549 T	*Euonymus japonicus*	China	OQ344763	OQ410582	OQ410584
P. chaoyangensis	CFCC 58805	*Euonymus japonicus*	China	OQ344764	OQ410583	OQ410585
P. chiangmaiensis	MFLUCC 22–0127	*Phyllostachys edulis*	Thailand	OP497990	OP753374	OP752137
P. chiaroscuro	BRIP 72970 T	*Sporobolus natalensis*	Australia	OK422510	OK423753	OK423752
P. chinensis	MFLUCC 12–0273 T	NA	China	JX398995	–	–
P. clavata	MFLUCC 12–0268 T	*Buxus* sp.	China	JX398990	JX399056	JX399025
P. colombiensis	CBS 118553 T	*Eucalyptus urograndis*	Colombia	KM199307	KM199488	KM199421
P. cratoxyli	CGMCC 3.23512 T	*Cratoxylum cochinchinense*	China	OR248005	OR361480	OR381080
P. cratoxyli	LC8772	*Cratoxylum cochinchinense*	China	OR248004	OR361479	OR381079
P. cyclobalanopsidis	CFCC 54328 T	*Cyclobalanopsis glauca*	China	OK339735	OK358496	OK358511
P. cyclobalanopsidis	CFCC 55891	*Cyclobalanopsis glauca*	China	OK339736	OK358497	OK358512
P. cyclosora	**HJAUP C1724.221 T**	***Cyclosorus interruptus***	**China**	**PP962279**	**PP952247**	**PP952221**
P. cyclosora	**HJAUP C1724.222**	***Cyclosorus interruptus***	**China**	**PP962280**	**PP952246**	**PP952222**
P. cyclosora	**HJAUP C1725.221**	***Microlepia marginata***	**China**	**PP962281**	**PP952245**	**PP952223**
P. cyclosora	**HJAUP C1725.222**	***Microlepia marginata***	**China**	**PP962282**	**PP952244**	**PP952233**
P. cyclosora	**HJAUP C1726.221**	***Punica granatum***	**China**	**PP962283**	**PP952243**	**PP952224**
P. cyclosora	**HJAUP C1726.222**	***Punica granatum***	**China**	**PP962284**	**PP952242**	**PP952232**
P. daliensis	CGMCC 3.23548 T	*Rhododendron decorum*	China	OP082429	OP185511	OP185518
P. dianellae	CBS 143421 T	*Dianella* sp.	Australia	MG386051	–	MG386164
P. digitalis	MFLU 14–0208 T	*Digitalis purpurea*	New Zealand	KP781879	–	KP781883
P. dilucida	LC3232 T	*Camellia sinensis*	China	KX894961	KX895178	KX895293
P. dilucida	LC8184	*Camellia sinensis*	China	KY464138	KY464148	KY464158
P. diploclisiae	CBS 115449	*Psychotria tutcheri*	China	KM199314	KM199485	KM199416
P. diploclisiae	CBS 115587 T	*Diploclisia glaucescens*	China	KM199320	KM199486	KM199419
P. disseminata	CBS 143904	*Persea americana*	New Zealand	MH554152	MH554587	MH554825
P. disseminata	MEAN 1165	*Pinus pinea*	Portugal	MT374687	MT374699	MT374712
P. diversiseta	MFLUCC 12–0287 T	*Rhododendron* sp.	China	JX399009	JX399073	JX399040
P. doitungensis	MFLUCC 14–0115	*Dendrobium* sp.	Thailand	MK993574	MK975832	MK975837
P. dracaenae	HGUP 4037 T	*Dracaena fragrans*	China	–	MT598644	MT598645
P. dracaenicola	MFLUCC 18–0913 T	*Dracaena* sp.	Thailand	MN962731	MN962732	MN962733
P. dracaenicola	MFLUCC 18–0914	*Dracaena* sp.	Thailand	MN962734	MN962735	MN962736
P. dracontomelonis	MFLU 14–0207	*Dracontomelon dao*	Thailand	KP781877	KP781880	–
P. eleuthero–cocci	HMJAU 60189	*Eleutherococcus brachypus*	China	OL996126	–	–
P. eleuthero–cocci	HMJAU 60190	*Eleutherococcus brachypus*	China	OL996127	–	OL898722
P. endophytica	MFLUCC 18–0932 T	*Magnolia garrettii*	Thailand	MW263946	MW417119	–
P. endophytica	MFLUCC 18–0946	*Magnolia garrettii*	Thailand	MW263947	MW729384	–
P. ericacearum	IFRDCC 2439 T	*Rhododendron delavayi*	China	KC537807	KC537814	KC537821
P. eriobotryae	**HJAUP C1742.221 T**	***Eriobotrya japonica***	**China**	**PP962289**	**PP952238**	**PP952227**
P. eriobotryae	**HJAUP C1742.222**	***Eriobotrya japonica***	**China**	**PP962291**	**PP952237**	**PP952228**
P. etonensis	BRIP 66615 T	*Sporobolus jacquemontii*	Australia	MK966339	MK977635	MK977634
P. exudata	CGMCC 3.23488 T	*Aucuba japonica*	China	OR247985	OR361460	OR381060
P. exudata	LC15850	*Aucuba japonica*	China	OR247986	OR361461	OR381061
P. ficicrescens	HGUP 861 T	*Camellia japonica*	China	MZ477311	MZ868328	MZ868301
P. foliicola	CFCC 54440 T	*Castanopsis faberi*	China	ON007029	ON005046	ON005057
P. foliicola	CFCC 57359	*Castanopsis faberi*	China	ON007030	ON005047	ON005058
P. foliicola	CFCC 57360	*Castanopsis faberi*	China	ON007031	ON005048	ON005059
P. formosana	NTUCC 17–009 T	Poaceae sp.	China	MH809381	MH809389	MH809385

Species	Strain Number	Host/Substrate	Locality	GenBank Accession Number		
				ITS	*tef1-a*	*tub2*
P. formosana	NTUCC 17–010	Poaceae sp.	China	MH809382	MH809390	MH809386
P. furcata	MFLUCC 12–0054 T	*Camellia sinensis*	Thailand	JQ683724	JQ683740	JQ683708
P. furcata	LC6691	*Camellia sinensis*	China	KX895030	KX895248	KX895363
P. fusiformis	CGMCC 3.23495 T	*Rhododendron* sp.	China	OR247995	OR361470	OR381070
P. fusiformis	LC15852	*Rhododendron* sp.	China	OR247996	OR361471	OR381071
P. fusoidea	CGMCC 3.23545 T	*Rhododendron delavayi*	China	OP082427	OP185512	OP185519
P. ganzhouensis	CGMCC 3.23489 T	*Cinnamomum camphora*	China	OR247987	OR361462	OR381062
P. ganzhouensis	LC5089	*Cinnamomum camphora*	China	OR247998	OR361473	OR381073
P. gardeniae	**HJAUP C1729.221 T**	***Gardenia jasminoides***	**China**	**PP962285**	**PP952241**	**PP952225**
P. gardeniae	**HJAUP C1729.222**	***Gardenia jasminoides***	**China**	**PP962286**	**PP952240**	**PP952226**
P. gardeniae	**HJAUP C1729.223**	***Gardenia jasminoides***	**China**	**PP962287**	**PP952239**	**PP952231**
P. gaultheriae	IFRD 411–014 T	*Gaultheria forrestii*	China	KC537805	KC537812	KC537819
P. gibbosa	NOF 3175 T	*Gaultheria shallon*	Canada	LC311589	LC311591	LC311590
P. grevilleae	CBS 114127 T	*Grevillea* sp.	Australia	KM199300	KM199504	KM199407
P. guangdongensis	ZHKUCC 22–0016 T	*Arenga pinnata*	China	ON180762	ON221520	ON221548
P. guangdongensis	ZHKUCC 22–0017	*Arenga pinnata*	China	ON180763	ON221521	ON221549
P. guangdongensis	ZHKUCC 22–0018	*Arenga pinnata*	China	ON180764	ON221522	ON221550
P. guangxiensis	CFCC 54308 T	*Quercus griffithii*	China	OK339737	OK358498	OK358513
P. guangxiensis	CFCC 54300	*Quercus griffithii*	China	OK339738	OK358499	OK358514
P. guiyangensis	CFCC 70626	*Eriobotrya japonicac*	China	PP784740	PP842629	PP842617
P. guiyangensis	CFCC 70630	*Rohdea japonica*	China	PP784741	PP842630	PP842618
P. guizhouensis	CFCC 54803	*Cyclobalanopsis glauca*	China	ON007035	ON005052	ON005063
P. guizhouensis	CFCC 57364 T	*Cyclobalanopsis glauca*	China	ON007036	ON005053	ON005064
P. hawaiiensis	CBS 114491 T	*Leucospermum* sp.	USA	KM199339	KM199514	KM199428
P. hederae	**HJAUP C1638.221 T**	***Hedera helix***	**China**	**PP962270**	**PP952252**	**PP952234**
P. hederae	**HJAUP C1638.222**	***Hedera helix***	**China**	**PP962271**	**–**	**PP952216**
P. hispanica	CBS 115391 T	*Protea* sp.	Spain	MH553981	MH554399	MH554640
P. hollandica	CBS 265.33 T	*Sciadopitys verticillata*	Netherlands	KM199328	KM199481	KM199388
P. hollandica	MEAN 1091 T	*Pinus pinea*	Portugal	MT374678	MT374691	MT374703
P. humicola	CBS 336.97 T	Soil	Papua New Guinea	KM199317	KM199484	KM199420
P. hunanensis	CSUFTCC15 T	*Camellia oleifera*	China	OK493599	OK507969	OK562374
P. hunanensis	CSUFTCC18	*Camellia oleifera*	China	OK493600	OK507970	OK562375
P. hydei	MFLUCC 20–0135 T	*Litsea petiolata*	Thailand	MW266063	MW251113	MW251112
P. iberica	CAA 1004 T	*Pinus radiata*	Spain	MW732248	MW759038	MW759035
P. iberica	CAA 1006	*Pinus radiata*	Spain	MW732249	MW759039	MW759036
P. inflexa	MFLUCC 12–0270 T	Unidentified tree	China	JX399008	JX399072	JX399039
P. intermedia	MFLUCC 12–0259 T	Unidentified tree	China	JX398993	JX399059	JX399028
P. italiana	MFLU 14–0214 T	*Cupressus glabra*	Italy	KP781878	KP781881	KP781882
P. jesteri	MFLUCC12–0279	*Fagraea bodenii*	China	JX399012	JX399076	JX399043
P. jiangsuensis	CFCC 59538	*Pinus massoniana*	China	OR533577	OR539186	OR539191
P. jiangsuensis	CFCC 59539	*Pinus massoniana*	China	OR533578	OR539187	OR539192
P. jiangsuensis	CFCC 59542	*Pinus massoniana*	China	OR533581	OR539190	OR539195
P. jiangxiensis	LC4399 T	*Camellia* sp.	China	KX895009	KX895227	KX895341
P. jiangxiensis	LC4242	*Eurya* sp.	China	KX895035	KX895213	KX895327
P. jinchanghensis	LC6636 T	*Camellia sinensis*	China	KX895028	KX895247	KX895361
P. jinchanghensis	LC8190	*Camellia sinensis*	China	KY464144	KY464154	KY464164
P. kaki	KNU–PT–1804 T	*Diospyros kaki*	Korea	LC552953	LC553555	LC552954
P. kandelicola	NCYUCC 19–0355 T	*Kandelia candel*	China	MT560723	MT563102	MT563100

Species	Strain Number	Host/Substrate	Locality	GenBank Accession Number		
				ITS	*tef1-a*	*tub2*
P. kenyana	CBS 442.67 T	*Coffea* sp.	Kenya	KM199302	KM199502	KM199395
P. kenyana	LC6633	*Camellia sinensis*	China	KX895027	KX895246	KX895360
P. kenyana	CFCC 54962	*Quercus aliena*	China	OM746237	OM840009	OM839910
P. kenyana	CFCC 54805	*Cyclobalanopsis glauca*	China	OM746253	OM840025	OM839926
P. kenyana	CFCC 55088	*Castanopsis fissa*	China	OM746254	OM840026	OM839927
P. knightiae	CBS 111963	*Knightia* sp.	New Zealand	KM199311	KM199495	KM199406
P. knightiae	CBS 114138 T	*Knightia* sp.	New Zealand	KM199310	KM199497	KM199408
P. krabiensis	MFLUCC 16–0260 T	*Pandanus* sp.	Thailand	MH388360	MH388395	MH412722
P. leucadendri	CBS 121417 T	*Leucadendron* sp.	South Africa	MH553987	MH554412	MH554654
P. licualicola	HGUP 4057 T	*Licuala grandis*	China	KC492509	KC481684	KC481683
P. lijiangensis	CFCC 50738 T	*Castanopsis carlesii* var. *spinulosa*	China	KU860520	KU844185	KU844184
P. linearis	MFLUCC 12–0271 T	*Trachelospermum* sp.	China	JX398992	JX399058	JX399027
P. linguae	ZHKUCC 22–0159 T	*Pyrrosia lingua*	China	OP094104	OP186110	OP186108
P. linguae	ZHKUCC 22–0160	*Pyrrosia lingua*	China	OP094103	OP186109	OP186107
P. lithocarpi	CFCC 55100 T	*Lithocarpus chiungchungensis*	China	OK339742	OK358503	OK358518
P. lithocarpi	CFCC 55893	*Lithocarpus chiungchungensis*	China	OK339743	OK358504	OK358519
P. lobata	CGMCC 3.23467 T	*Lithocarpus glaber*	China	OR247976	OR361451	OR381051
P. lobata	LC15843	*Lithocarpus glaber*	China	OR247977	OR361452	OR381052
P. loeiana	MFLUCC 22–0123 T	Dead leaves	Thailand	OP497988	OP737881	OP713769
P. longiappendiculata	LC3013	*Camellia sinensis*	China	KX894939	KX895156	KX895271
P. lushanensis	LC4344 T	*Camellia* sp.	China	KX895005	KX895223	KX895337
P. lushanensis	LC8182	*Camellia* sp.	China	KY464136	KY464146	KY464156
P. lushanensis	LC8183	*Camellia* sp.	China	KY464137	KY464147	KY464157
P. lushanensis	CFCC 54894	*Quercus serrata*	China	OM746282	OM840054	OM839955
P. macadamiae	BRIP 63738b T	*Macadamia integrifolia*	Australia	KX186588	KX186621	KX186680
P. macadamiae	BRIP 63739b	*Macadamia integrifolia*	Australia	KX186587	KX186620	KX186679
P. macadamiae	BRIP 637441a	*Macadamia integrifolia*	Australia	KX186586	KX186619	KX186678
P. machili	CGMCC 3.23511 T	*Machilus* sp.	China	OR248003	OR361478	OR381078
P. machiliana	**HJAUP C1790.221 T**	***Machilus pauhoi***	**China**	**PP962355**	**PP952253**	**PP952214**
P. machiliana	**HJAUP C1790.222**	***Machilus pauhoi***	**China**	**PP962356**	**PP952254**	**PP952215**
P. machiliana	**HJAUP C1704.221**	***Rhododendron simsii***	**China**	**PP962276**	**PP952255**	**PP952211**
P. machiliana	**HJAUP C1704.222**	***Rhododendron simsii***	**China**	**PP962277**	**PP952256**	**PP952212**
P. machiliana	**HJAUP C1704.223**	***Rhododendron simsii***	**China**	**PP962278**	**PP952257**	**PP952213**
P. malayana	CBS 102220	*Macaranga triloba*	Malaysia	KM199306	KM199482	KM199411
P. mangifericola	**HJAUP C1639.221 T**	***Mangifera indica***	**China**	**PP962272**	**PP952251**	**PP952217**
P. mangifericola	**HJAUP C1639.222**	***Mangifera indica***	**China**	**PP962273**	**PP952250**	**PP952218**
P. manyueyuanani	NTUPPMCC 18-165 T	*Ophiocordyceps* sp.	China	OR125060	OR126313	OR126306
P. manyueyuanani	NTUPPMCC 22-012	*Ophiocordyceps* sp.	China	OR125061	OR126314	OR126307
P. menhaiensis	YN3A1 T	*Camellia sinensis*	China	KU252272	KU252401	KU252488
P. monochaeta	CBS 144.97 T	*Quercus robur*	Netherlands	KM199327	KM199479	KM199386
P. monochaeta	CBS 440.83	*Taxus baccata*	Netherlands	KM199329	KM199480	KM199387
P. multiappendiculata	CGMCC 3.23514 T	NA	China	OR248008	OR361483	OR381083
P. multicolor	CFCC59981 T	*Taxus chinensis*	China	OQ626676	OQ714341	OQ714336
P. multicolor	CFCC59982	*Taxus chinensis*	China	OQ771896	OQ779483	OQ779488
P. nanjingensis	CSUFTCC20	*Camellia oleifera*	China	OK493603	OK507973	OK562378
P. nanjingensis	CSUFTCC04	*Camellia oleifera*	China	OK493604	OK507974	OK562379
P. nanningensis	CSUFTCC10 T	*Camellia oleifera*	China	OK493596	OK507966	OK562371

Species	Strain Number	Host/Substrate	Locality	GenBank Accession Number		
				ITS	*tef1-a*	*tub2*
P. nanningensis	CSUFTCC11	*Camellia oleifera*	China	OK493597	OK507967	OK562372
P. nannuoensis	SAUCC232203 T	Unknown host	China	OR733504	OR912991	OR863909
P. nannuoensis	SAUCC232204	Unknown host	China	OR733503	OR912992	OR863910
P. neglecta	TAP1100 T	*Quercus myrsinaefolia*	Japan	AB482220	LC311600	LC311599
P. neolitseae	NTUCC 17–011 T	*Neolitsea villosa*	Taiwan	MH809383	MH809391	MH809387
P. neolitseae	CFCC 54590	*Lithocarpus amygdalifolius*	China	OK339744	OK358505	OK358520
P. novae-hollandiae	CBS 130973 T	*Banksia grandis*	Australia	KM199337	KM199511	KM199425
P. oryzae	CBS 111522	*Telopea* sp.	USA	KM199294	KM199493	KM199394
P. oryzae	CBS 171.26	NA	Italy	KM199304	KM199494	KM199397
P. oryzae	CBS 353.69 T	*Oryza sativa*	Denmark	KM199299	KM199496	KM199398
P. pallidotheae	MAFF 240993 T	*Pieris japonica*	Japan	AB482220	LC311585	LC311584
P. pandanicola	MFLUCC 16–0255 T	*Pandanus* sp.	Thailand	MH388361	MH388396	MH412723
P. papuana	CBS 331.96 T	Coastal soil	Papua New Guinea	KM199321	KM199491	KM199413
P. papuana	CBS 887.96	*Cocos nucifera*	Papua New Guinea	KM199318	KM199492	KM199415
P. parva	CBS 265.37	*Delonix regia*	NA	KM199312	KM199508	KM199404
P. parva	CBS 278.35 T	*Leucothoe fontanesiana*	NA	KM199313	KM199509	KM199405
P. photinicola	GZCC 16–0028 T	*Photinia serrulata*	China	KY092404	KY047662	KY047663
P. phyllostachydis	ZHKUCC 23–0873 T	NA	China	OR343210	OR367675	OR367676
P. pini	MEAN 1092 T	*Pinus pinea*	Portugal	MT374680	MT374693	MT374705
P. pinicola	KUMCC 19–0183 T	*Pinus armandii*	China	MN412636	MN417509	MN417507
P. piraubensis	COAD 2165 T	*Psidium guajava*	Brazil	MH627381	MH643774	MH643773
P. portugalica	CBS 393.48 T	NA	Portugal	KM199335	KM199510	KM199422
P. pruni	CGMCC 3.23507 T	*Prunus cerasoides*	China	OR248001	OR361476	OR381076
P. pruni	LC15860	*Prunus cerasoides*	China	OR248002	OR361477	OR381077
P. rhaphiolepis	SAUCC367701 T	*Rhaphiolepis indica*	China	OR733502	OR912994	OR863906
P. rhaphiolepis	SAUCC367702	*Rhaphiolepis indica*	China	OR733501	OR912995	OR863907
P. rhizophorae	MFLUCC 17–0416 T	*Rhizophora mucronata*	Thailand	MK764283	MK764327	MK764349
P. rhizophorae	MFLUCC 17–0417	*Rhizophora mucronata*	Thailand	MK764284	MK764328	MK764350
P. rhododendri	IFRDCC 2399 T	*Rhododendron sinogrande*	China	KC537804	KC537811	KC537818
P. rhodomyrtus	CFCC 54733	*Quercus aliena*	China	OM746310	OM840082	OM839983
P. rhodomyrtus	CFCC 55052	*Cyclobalanopsis augustinii*	China	OM746311	OM840083	OM839984
P. rosarioides	CGMCC 3.23549 T	*Rhododendron decorum*	China	OP082430	OP185513	OP185520
P. rosea	MFLUCC 12–0258 T	*Pinus* sp.	China	JX399005	JX399069	JX399036
P. rubrae	CGMCC 3.23499 T	*Quercus rubra*	China	OR247997	OR361472	OR381072
P. rubrae	LC8233	*Plagiogyria glauca*	China	OR248000	OR361475	OR381075
P. sabal	ZHKUCC 22–0027	*Sabal mexicana*	China	ON180765	ON221523	ON221551
P. sabal	ZHKUCC 22–0029	*Sabal mexicana*	China	ON180767	ON221525	ON221553
P. scoparia	CBS 176.25 T	*Chamaecyparis* sp.	China	KM199330	KM199478	KM199393
P. sequoiae	MFLUCC 13–0399 T	*Sequoia sempervirens*	Italy	KX572339	–	–
P. shaanxiensis	CFCC 54958 T	*Quercus variabilis*	China	ON007026	ON005043	ON005054
P. shaanxiensis	CFCC 57356	*Quercus variabilis*	China	ON007027	ON005044	ON005055
P. shandogensis	JZB340038 T	*Robinia pseudoacacia*	China	MN625275	MN626740	MN626729
P. shorea	MFLUCC 12–0314 T	*Shorea obtusa*	Thailand	KJ503811	KJ503817	KJ503814
P. sichuanensis	SC3A21 T	*Camellia sinensis*	China	KX146689	KX146748	KX146807
P. silvicola	CFCC 55296 T	*Cyclobalanopsis kerrii*	China	ON007032	ON005049	ON005060
P. silvicola	CFCC 54915	*Cyclobalanopsis kerrii*	China	ON007033	ON005050	ON005061
P. silvicola	CFCC 57363	*Cyclobalanopsis kerrii*	China	ON007034	ON005051	ON005062

Species	Strain Number	Host/Substrate	Locality	GenBank Accession Number		
				ITS	*tef1-α*	*tub2*
P. smilacicola	MFLUCC 22–0124	*Smilax china*	Thailand	OP497989	OP737879	OP762674
P. smilacicola	MFLUCC 22–0125 T	*Dioscorea* sp.	Thailand	OP497991	OP753376	OP762673
P. sonneratiae	CFCC 57392	*Sonneratia apetala*	China	ON114182	ON086810	ON086814
P. sonneratiae	CFCC 57394 T	*Sonneratia apetala*	China	ON114184	ON086812	ON086816
P. sonneratiae	CFCC 57395	*Sonneratia apetala*	China	ON114185	ON086813	ON086817
P. spathulata	CBS 356.86 T	*Gevuina avellana*	Chile	KM199338	KM199513	KM199423
P. spathuliappendiculata	CBS 144035 T	*Phoenix canariensis*	Australia	MH554172	MH554607	MH554845
P. suae	CGMCC 3.23546 T	*Rhododendron delavayi*	China	OP082428	OP185514	OP185521
P. taxicola	CFCC59976 T	*Taxus chinensis*	China	OQ626673	OQ714338	OQ714333
P. taxicola	CFCC59978	*Taxus chinensis*	China	OQ771893	OQ779480	OQ779485
P. telopeae	CBS 114137	*Protea* sp.	Australia	KM199301	KM199559	KM199469
P. telopeae	CBS 114161 T	*Telopea* sp.	Australia	KM199296	KM199500	KM199403
P. telopeae	CBS 113606	*Telopea* sp.	Australia	KM199295	KM199498	KM199402
P. terricola	CBS 141.69 T	Soil	Pacific Islands	MH554004	MH554438	MH554680
P. thailandica	MFLUCC 17–1616 T	*Rhizophora apiculata*	Thailand	MK764286	MK764330	MK764352
P. thailandica	MFLUCC 17–1617	*Rhizophora apiculata*	Thailand	MK764285	MK764329	MK764351
P. trachycarpicola	OP068 T	*Trachycarpus fortunei*	China	JQ845947	JQ845946	JQ845945
P. trachycarpicola	IFRDCC 2403	*Podocarpus macrophyllus*	China	KC537809	KC537816	KC537823
P. trachycarpicola	LC4523	*Camellia sinensis*	China	KX895011	KX895230	KX895344
P. tumida	CFCC 55158 T	*Rosa chinensis*	China	OK560610	OL814524	OM158174
P. tumida	CFCC 55159	*Rosa chinensis*	China	OK560613	OL814527	OM158177
P. tumida	CGMCC 3.23502	NA	China	OR247999	OR361474	OR381074
P. unicolor	MFLUCC 12–0276 T	*Rhododendron* sp.	China	JX398999	–	JX399030
P. unicolor	MFLUCC 12–0275	Unidentified tree	China	JX398998	JX399063	JX399029
P. verruculosa	MFLUCC 12–0274 T	*Rhododendron* sp.	China	JX398996	JX399061	–
P. wulichongensis	CGMCC 3.23469 T	Poaceae	China	OR247978	OR361453	OR381053
P. wulichongensis	LC15846	Poaceae	China	OR247979	OR361454	OR381054
P. yanglingensis	LC 4553 T	*Camellia sinensis*	China	KX895012	KX895231	KX895345
P. yanglingensis	LC 3412	*Camellia sinensis*	China	KX894980	KX895197	KX895312
P. yunnanensis	HMAS 96359 T	*Podocarpus macrophyllus*	China	AY373375	–	–
Nonappendiculata quercina	CBS 116061 T	*Quercus suber*	Italy	MH553982	MH554400	MH554641
N. quercina	CBS 270.82	*Quercus pubescens*	Italy	MH554025	MH554459	MH554701

T = ex–type culture. **BRIP** = Queensland Plant Pathology Herbarium, Brisbane, Australia; **CAA** = Culture collection of Artur Alves, housed at Department of Biology, University of Aveiro, Aveiro, Portugal; **CBS** = culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CFCC** = China Forestry Culture Collection Center, China; **CGMCC** = China General Microbiological Culture Collection Center, Beijing, China; **ICMP** = International Collection of Microorganisms from Plants, Auckland, New Zealand; **CSUFTCC** = Central South University of Forestry and Technology Culture Collection, Hunan, China; **GZCC** = Guizhou Academy of Agricultural Sciences Culture Collection, Guizhou, China; **HGUP** = Plant Pathology Herbarium of Guizhou University, Guizhou, China; **HMAS** = Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. **HMJAU** = Herbarium of Mycology of Jilin Agricultural University, Jilin, China; **SAUCC** = Shandong Agricultural University Culture Collection, Taian, Shandong, China; **ICMP** = International Collection of Microorganisms from Plants, Auckland, New Zealand; **IFRDCC** = International Fungal Research and Development Culture Collection, Kunming, Yunnan China; **KNU** = Kyungpook National University, Daegu, South Korea; **KUMCC** = Kunming Institute of Botany Culture Collection, Yunnan, China; **LC** = working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; **MAFF** = Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; **MEAN** = Instituto Nacional de Investigação Agrária e Veterinária I. P.; **MFLU** = Mae Fah Luang University Herbarium, Thailand; **MFLUCC** = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NCYUCC** = The National Chiayi University Culture Collection, Jiayi, Taiwan; **NOF** = The Fungus Culture Collection of the Northern Forestry Centre, Alberta, Canada; **NTUCC** = The Department of Plant Pathology and Microbiology, National Taiwan University Culture Collection, Taipei, Taiwan China; **TAP** = Tamagawa University, Tokyo, Japan; **ZHKUCC** = the culture collection of Zhongkai University of Agriculture and Engineering, Guangzhou City, Guangdong, China; ITS = internal transcribed spacer; *tub2* = β–tubulin; *tef1-α* = translation elongation factor1–α.

Phylogenetic analyses

The newly sequences generated in this study were analyzed with other related sequences obtained from GenBank (Table 2), based on recent publications (Hsu et al. 2024; Li et al. 2024; Wang et al. 2024; Zhao et al. 2024). *Nonappendiculata quercina* (CBS 116061) and *N. quercina* (CBS 270.82) were used as outgroup taxa. Multiple sequences were aligned using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/index.html) with default settings (Katoh and Standley 2013). To identify *Pestalotiopsis* taxa, single gene phylogenies were inferred for ITS, *tef1-α* and *tub2*, and the sequences of three loci (ITS, *tef1-α* and *tub2*) were concatenated using the "Concatenate Sequence" function in Phylosuite software v1.2.1 (Zhang et al. 2020) to conduct a multi-locus analysis including maximum-likelihood (ML) and Bayesian inference (BI) methods, and the best evolutionary model was selected for each alignment dataset using ModelFinder (Kalyaanamoorthy et al. 2017) and incorporated into the analyses. For the ML analysis, maximum-likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under best partitioned models, and tree stability was evaluated with 10,000 ultrafast bootstraps (Minh et al. 2013). The TIM3e+I+G4 model was selected as the most suitable for ITS data partitions, and the TIM2+F+I+G4 model was selected for *tef1-α* and *tub2* data partition. For the BI analysis, Bayesian inference phylogenies were performed using MrBayes 3.2.6 (Ronquist et al. 2012), in which the best nucleotide substitution model for each locus was identified using ModelFinder of Phylosuite, and the best-fit model was GTR+F+I+G4 for ITS, *tef1-α* and *tub2*. Phylogenetic trees were visualized in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree, accessed on 12 September 2024), edited and typeset using Adobe Illustrator 2021. The names of the isolates from the present study are marked in red in the trees.

Results

Molecular phylogeny

To identify the isolated *Pestalotiopsis* strains, the ITS sequence data were used for initial identification in the present study. By the BLASTn analysis of ITS sequence, 24 strains were categorised into the genus *Pestalotiopsis*. Subsequently, based on maximum-likelihood (ML) and Bayesian inference (BI), the combined analysis of ITS, *tef1-α* and *tub2* gene data was used to construct phylogenetic trees for further determination of the phylogenetic position of these strains. The phylogenetic results represented by the best-scoring ML consensus tree (lnL = –14416.332) are shown in Fig. 1. The 24 isolates obtained from different plants in our study nested within the known *Pestalotiopsis* species with reliable support values. In the multi-loci phylogenies of ITS, *tef1-α* and *tub2*, a total of 266 strains representing 147 accepted species were comprised in the final alignment matrix of *Pestalotiopsis*. *Nonappendiculata quercina* (CBS 116061) and *N. quercina* (CBS 270.82) served as outgroups. The combined data set (ITS: 1–510, *tef1-α*: 511–891 and *tub2*: 892–1284) was composed of 684 distinct patterns, 468 parsimony informative sites, 103 singleton sites, and 713 constant sites. A total of three single-locus data sets, ITS, *tef1-α* and *tub2*, contained 107, 181 and 180 parsimony informative sites, respectively.

Figure 1. Phylogenetic relationship of *Pestalotiopsis* based on concatenated sequences of ITS, *tef1-α* and *tub2* sequence data. The ML and BI bootstrap support values above 80% and 0.80 are given above the nodes. Bar = 0.03 substitution per nucleotide position. The tree is rooted to *Nonappendiculata quercina* (CBS 116061) and *N. quercina* (CBS 270.82). The strains from the present study are marked in red. Some branches are shortened according to the indicated multipliers to fit the page size, and these are indicated by the symbol (//).

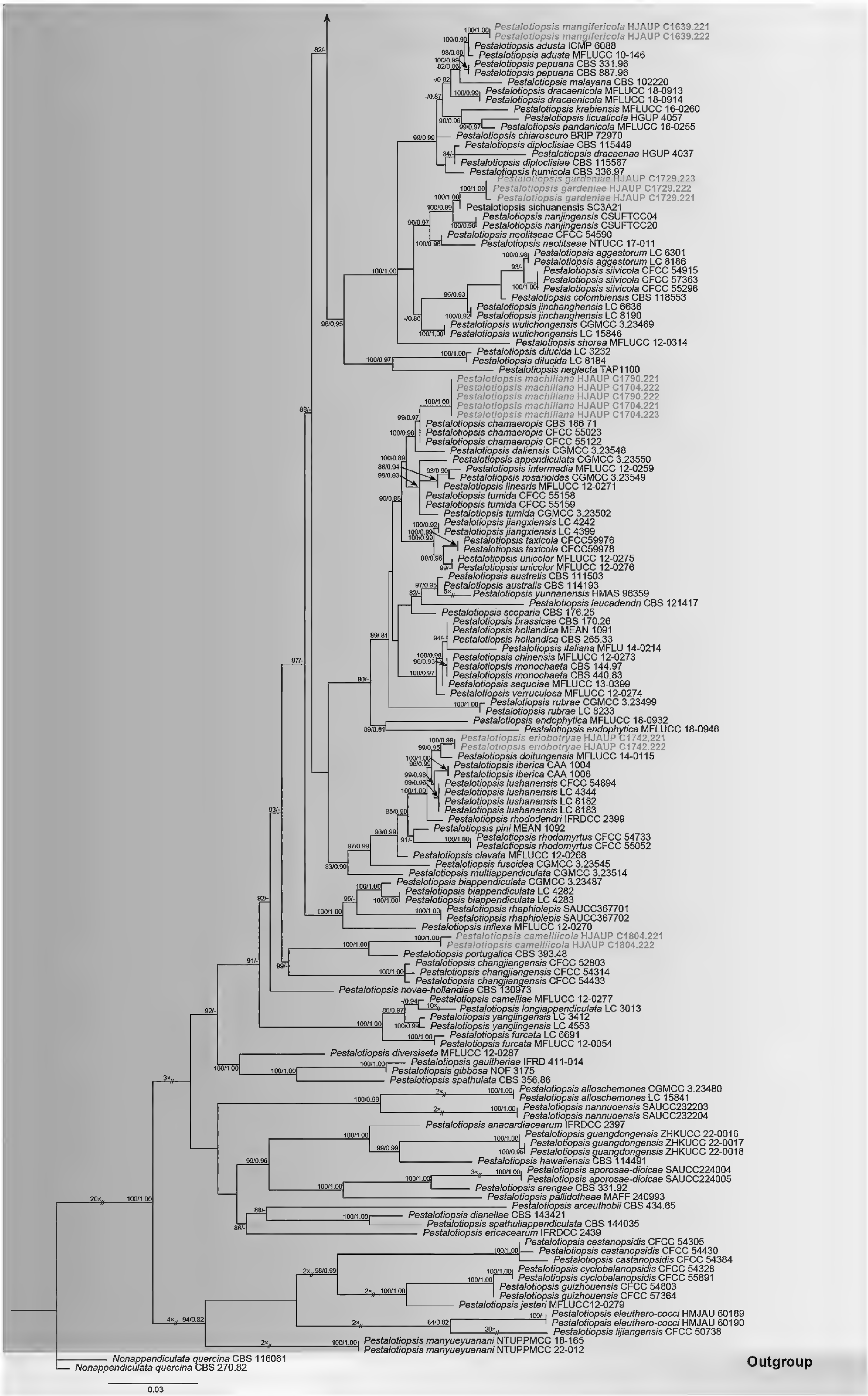

Figure 1. Continued.

Combining morphological characteristics and molecular phylogenetic analyses, the 24 strains in this study were introduced as eight new species, namely *Pestalotiopsis alpinicola*, *P. camelliicola*, *P. cyclosora*, *P. eriobotryae*, *P. gardeniae*, *P. hederae*, *P. machiliana* and *P. mangifericola*.

Taxonomy

***Pestalotiopsis alpinicola* X.X. Luo & Jian Ma, sp. nov.**
Index Fungorum: IF902319
Fig. 2

Type. China • Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun Town, Tropical Botanical Garden, on diseased leaves of *Alpinia zerumbet*, 23 June 2022, X.X. Luo (holotype HJAUP M1644.221; ex-type living culture HJAUP C1644.221).

Etymology. Referring to the host genus, *Alpinia* from which it was collected.

Description. Leaf tip blight and irregular pallid leaf spots. Asexual morph on PDA: Conidiomata acervular, globose, 710–1110 μm diam., solitary or aggregated in clusters, black. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform. Conidia fusiform, straight or slightly curved, 18.1–21.8 × 4.7–5.9 μm ($\bar{x}$ = 19.7 × 5.5 μm, n = 50), 4-septate, slightly constricted at the septa; basal cell conical, 2.6–4.4 μm ($\bar{x}$ = 3.6 μm) long, hyaline or sometimes pale brown, smooth, thin-walled, with a single filiform appendage, unbranched, 3.6–6.2 μm ($\bar{x}$ = 5.1 μm) long; three median cells doliiform to cylindrical, smooth, 10–13 μm ($\bar{x}$ = 12 μm) long, concolorous or sometimes darker at the two upper cells, somewhat constricted at the septa, second cell from the base pale brown to brown, 3.5–4.5 μm ($\bar{x}$ = 4.1 μm) long, third cell brown, 3.3–4.2 μm ($\bar{x}$ = 3.8 μm) long, fourth cell pale brown to brown, 3.6–4.5 μm ($\bar{x}$ = 4.1 μm) long; apical cell conical to acute, hyaline, smooth, thin-walled, 3.1–4.5 μm ($\bar{x}$ = 3.6 μm) long, with 1–3 (mostly 2) filiform appendages, arising from the apical crest, unbranched, 13.1–20.9 μm long. Sexual morph not observed.

Culture characteristics. Colonies on PDA grow fast, flat and spreading, growing all over the Petri dish after 2 weeks at 25 °C in darkness, white, with flocculent aerial mycelium and entire edge, forming black conidiomata, and reverse pale straw.

Additional specimen examined. China • Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun Town, Tropical Botanical Garden, 23 June 2022, X.X. Luo. On diseased leaves of *Alpinia zerumbet*; paratype HJAUP M1644.222, living culture HJAUP C1644.222.

Note. Two strains (HJAUP C1644.221 and HJAUP C1644.222) of *Pestalotiopsis alpinicola* isolated from leaf spots of *Alpinia zerumbet* clustered with *P. lithocarpi* (CFCC 55100 and CFCC 55893) with 95% ML/0.68 BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1644.221 is closely related to *P. lithocarpi* (CFCC 55100) and comparisons of their nucleotides showed 20 bp differences (2%, including zero gap) nucleotide differences in three loci. Moreover, *P. alpinicola* is morphologically distinguished from *P. lithocarpi* Ning Jiang by its smaller conidia (4.7–5.9 μm vs. 6–7 μm) with shorter three median cells (10–13 μm vs. 12.5–14 μm) and fewer apical appendages (1–2 vs. 3–4) (Jiang et al. 2022).

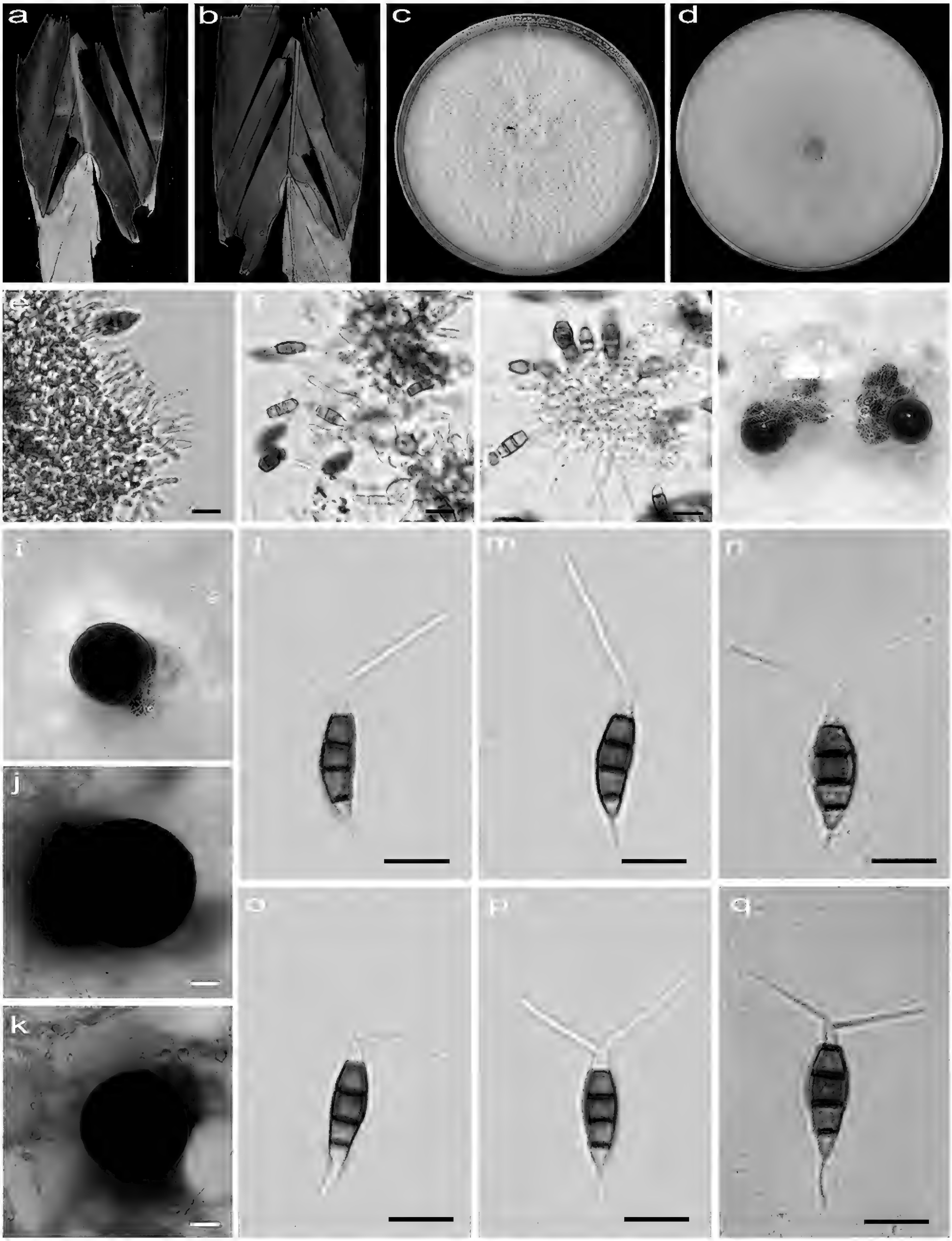

Figure 2. *Pestalotiopsis alpinicola* (HJAUP C1644.221, ex-type) **a, b** leaf of host plant (front and reverse) **c, d** culture on PDA (front and reverse) **e–g** conidiogenous cells and conidia **h–k** conidiomata **l–q** conidia. Scale bars: 200 μm (**j, k**); 10 μm (**e–g, l–q**).

Pestalotiopsis camelliicola **X.X. Luo & Jian Ma, sp. nov.**
Index Fungorum: IF902320
Fig. 3

Type. China • Jiangxi Province, Jingdezhen City, Changjiang District, Jingdezhen Botanical Garden, on diseased leaves of *Camellia japonica*, 3 November 2022, X.X. Luo (holotype HJAUP M1804.221; ex-type living culture HJAUP C1804.221).

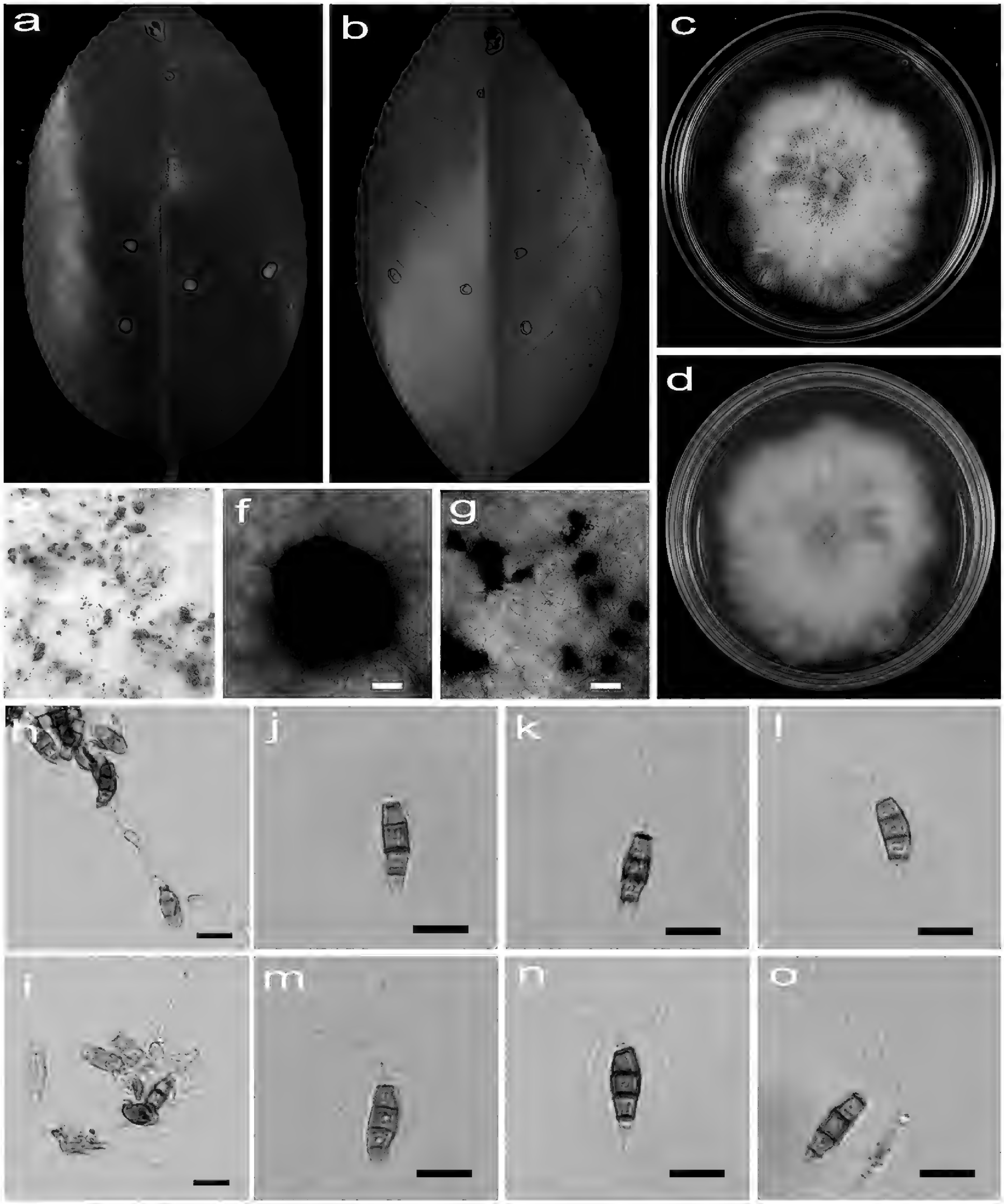

Figure 3. *Pestalotiopsis camelliicola* (HJAUP C1804.221, ex-type) **a, b** leaf of host plant (front and reverse) **c, d** culture on PDA (front and reverse) **e–g** conidiomata **h, i** conidiogenous cells and conidia **j–o** conidia. Scale bars: 200 µm (**f, g**); 10 µm (**h–o**).

Etymology. Referring to the host genus from which it was collected, *Camellia japonica*.

Description. Regular leaf spots, grey white in the center, and brown to dark brown at the margin. Asexual morph on PDA: Conidiomata acervular, 470–1320 μm diam., superficial, solitary or aggregated in clusters, dark brown. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform. Conidia fusiform, straight or slightly curved, 14.9–22.2 × 5.4–7.6 μm ($\bar{x}$ = 18.1 × 6.3 μm, n = 50), 4-septate, mostly with one minute guttules in each cell, slightly constricted at the septa; basal cell conical, 1.8–4 μm ($\bar{x}$ = 2.8 μm), pale brown, smooth, thin-walled, with a single filiform appendage, unbranched, 1.7–5.2 μm ($\bar{x}$ = 2.9 μm) long; three median cells doliiform to cylindrical, smooth, 11–14.4 μm ($\bar{x}$ = 12.4 μm), concolorous, pale brown to brown, somewhat constricted at the septa, second cell from the base 3.8–5.3 μm ($\bar{x}$ = 4.3 μm) long, third cell 3.6–4.7 μm ($\bar{x}$ = 4.2 μm) long, fourth cell 3.2–5 μm ($\bar{x}$ = 4 μm) long); apical cell conical to acute, hyaline, smooth, thin-walled, 2.2–3.8 μm ($\bar{x}$ = 2.9 μm) long, with 2–4 (mostly 3) filiform appendages, arising from the apical crest, branched, 9.5–20.3 μm ($\bar{x}$ = 12.4 μm) long. Sexual morph: not observed.

Culture characteristics. Colonies on PDA grow fast, filamentous, reaching 56–62 mm diam. after 5 days at 25 °C in darkness, white, with flocculent mycelium and entire edge, forming black, brown conidiomata, and reverse pale orange.

Additional specimen examined. CHINA • Jiangxi Province, Jingdezhen City, Changjiang District, Jingdezhen Botanical Garden, 3 November 2022, X.X. Luo. On diseased leaves of *Camellia japonica*, paratype HJAUP M1804.222, living culture HJAUP C1804.222.

Note. Two strains (HJAUP C1804.221 and HJAUP C1804.222) of *Pestalotiopsis camelliicola* isolated from leaf spots of *Camellia japonica* formed a distinct clade sister to *P. portugalica* (CBS 393.48) with 100% ML/1.00 BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1804.221 is closely related to *P. portugalica* (CBS 393.48) and comparisons of their nucleotides showed 20 bp differences (2%, including four gaps) nucleotide differences in three loci. Moreover, *P. camelliicola* is morphologically distinguished from *P. portugalica* Maharachch., K.D. Hyde & Crous in its solitary or scattered conidiomata and conidia with more apical filiform appendages (2–4 vs. 1–3). In addition, the conidia of *P. camelliicola* usually have one minute guttule at each cell, which are not observed in *P. portugalica* (Maharachchikumbura et al. 2014).

Pestalotiopsis cyclosora X.X. Luo & Jian Ma, sp. nov.

Index Fungorum: IF902321

Fig. 4

Type. CHINA • Jiangxi Province, Xinyu City, Yushui District, Baoshi Park, on diseased leaves of *Cyclosorus interruptus*, 2 November 2022, X.X. Luo (holotype HJAUP M1724.221; ex-type living culture HJAUP C1724.221).

Etymology. Referring to the host genus, *Cyclosorus* from which it was collected.

Description. Regular leaf spots, yellowish to grey white in the center, and dark brown at the margin. Asexual morph on PDA: Conidiomata acervular, globose, 460–780 μm diam., solitary, black. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform.

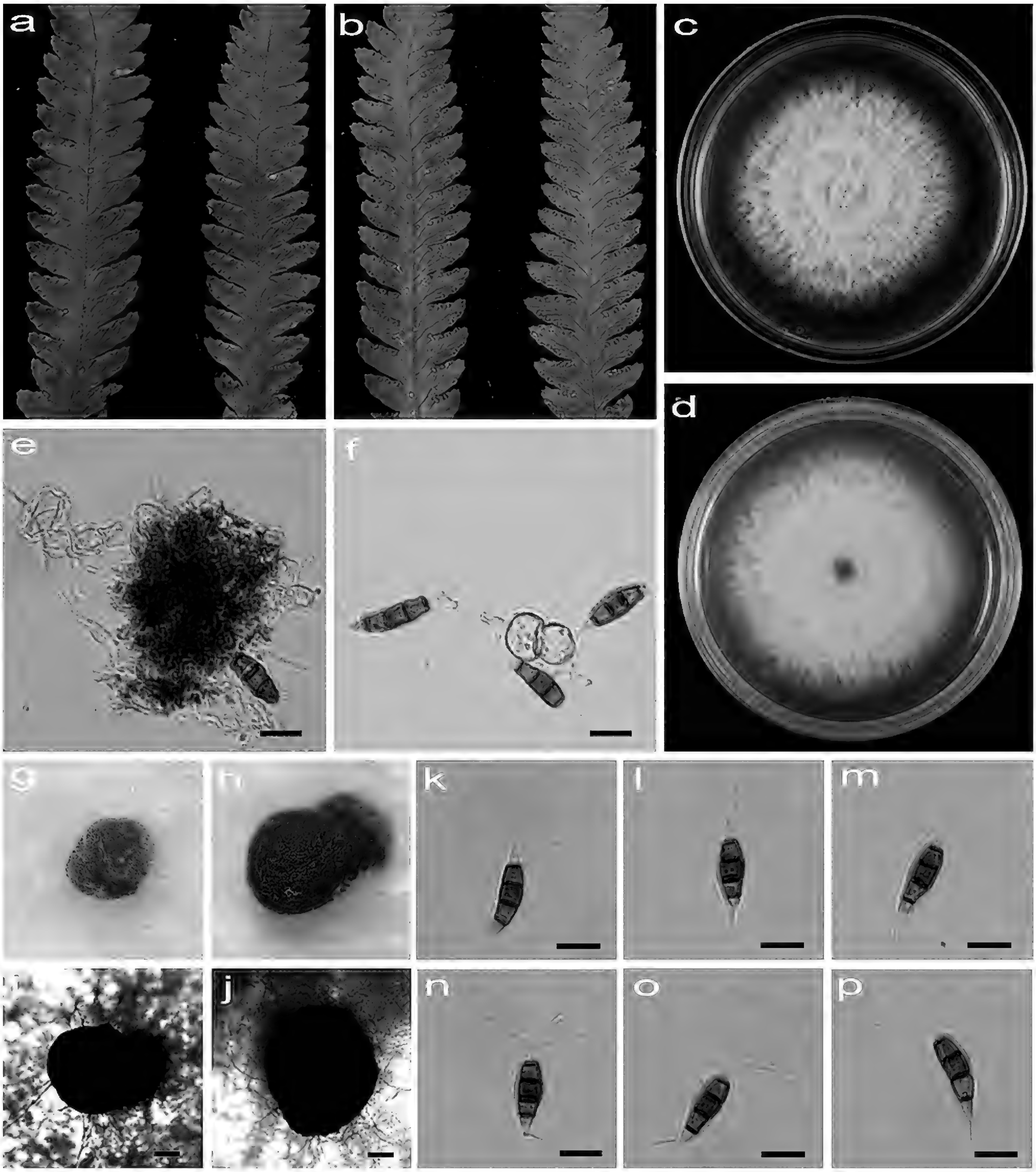

Figure 4. *Pestalotiopsis cyclosora* (HJAUP C1724.221) **a, b** leaf of host plant (front and reverse) **c, d** culture on PDA (front and reverse) **e, f** conidiogenous cells and conidia **g–j** conidiomata **k–p** conidia. Scale bars: 200 μm (**i, j**); 10 μm (**e, f, k–p**).

Conidia fusiform, straight or slightly curved, 16.3–26.1 × 5.4–7.1 μm ($\bar{x}$ = 21.3 × 6.4 μm, n = 50), 4-septate, slightly constricted at the septa; basal cell conical, 2.7–4.7 μm ($\bar{x}$ = 3.5 μm), hyaline or sometimes pale brown, smooth, thin-walled, with a single filiform appendage, unbranched, 4.1–10.6 μm ($\bar{x}$ = 7.7 μm) long; three median cells doliiform to cylindrical, smooth, 11–17.1 μm ($\bar{x}$ = 14.1 μm), concolorous or sometimes darker at the two upper cells, somewhat constricted at the septa, second cell from the base brown, 3.9–6.2 μm ($\bar{x}$ = 4.8 μm) long,

third cell brown to dark brown, 3.9–5.6 µm (x̄ = 4.7 µm) long, fourth cell brown, 3.8–5.7 µm (x̄ = 4.8 µm) long); apical cell conical to acute, hyaline, smooth, thin-walled, 2.6–4.2 µm (x̄ = 3.6 µm) long, with 1–4 (mostly 2 or 3) filiform appendages, arising from the apical crest, sometimes branched, 12.5–29.8 µm (x̄ = 20.1 µm) long. Sexual morph not observed.

Culture characteristics. Colonies on PDA grow fast, filamentous to circular, reaching 62–69 cm diam. after 5 days at 25 °C in darkness, regular edge, white, with filamentous aerial mycelium and entire edge, and reverse pale orange.

Additional specimen examined. China • Jiangxi Province, Xinyu City, Yushui District, Baoshi Park, 2 November 2022, X.X. Luo. On diseased leaves of *Cyclosorus interruptus*, paratype HJAUP M1724.222, living culture HJAUP C1724.222; on diseased leaves of *Microlepia marginata*, paratype HJAUP M1725.221, living culture HJAUP C1725.221; on diseased leaves of *Microlepia marginata*, paratype HJAUP M1725.222, living culture HJAUP C1725.222 • Yingtan City, Guixi County, Shangqing Town, Longhu Mountain National Forest Park, 3 November 2022, X.X. Luo. On diseased leaves of *Punica granatum*, paratype HJAUP M1726.221, living culture HJAUP C1726.221; on diseased leaves of *Punica granatum*, paratype HJAUP M1726.222, living culture HJAUP C1726.222.

Notes. Six strains (HJAUP C1724.221, HJAUP C1724.222, HJAUP C1725.221, HJAUP C1725.222, HJAUP C1726.221 and HJAUP C1726.222) of *Pestalotiopsis cyclosora* isolated from leaf spots of *Cyclosorus interruptus*, *Microlepia marginata* and *Punica granatum* clustered as a sister taxon to the clade containing *P. ficicrescens* (HGUP 861) and *P. biciliata* (CBS 124463 and CBS 236.38) with 90% ML/0.97 BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1724.221 is closely related to *P. ficicrescens* (HGUP 861) and *P. biciliata* (CBS 124463), and comparisons of their nucleotides showed 18 bp differences (2%, including three gaps) and 12 bp differences (1%, including two gaps) nucleotide differences in three loci, respectively. Moreover, *P. cyclosora* is morphologically distinguished from *P. ficicrescens* Qi Yang & Yong Wang bis in its conidia with darker median cells and longer filiform appendages at both ends (apical appendages: 12.5–29.8 µm vs. 10.5–18 µm, basal appendage: 4.1–10.6 µm vs. 3.5–7 µm), and more apical appendages (1–4 vs. 2–3) in apical cell (Hyde et al. 2023). *Pestalotiopsis cyclosora* is also different from *P. biciliata* Maharachch., K.D. Hyde & Crous, which has verruculose conidia with concolourous, olivaceous median cells and longer basal cell (4–7 µm vs. 2.7–4.7 µm) bearing two appendages (Maharachchikumbura et al. 2014).

***Pestalotiopsis eriobotryae* X.X. Luo & Jian Ma, sp. nov.**

Index Fungorum: IF902322
Fig. 5

Type. China • Jiangxi Province, Yingtan City, Guixi County, Shangqing Town, Longhu Mountain National Forest Park, on diseased leaves of *Eriobotrya japonica*, 3 November 2022, X.X. Luo (holotype HJAUP M1742.221; ex-type living culture HJAUP C1742.221).

Etymology. Referring to the host genus, *Eriobotrya* from which it was collected.

Description. Regular leaf spots, grey white in the center with black-spotted acervuli, and dark brown at the margin with rusty halo. Asexual morph on PDA:

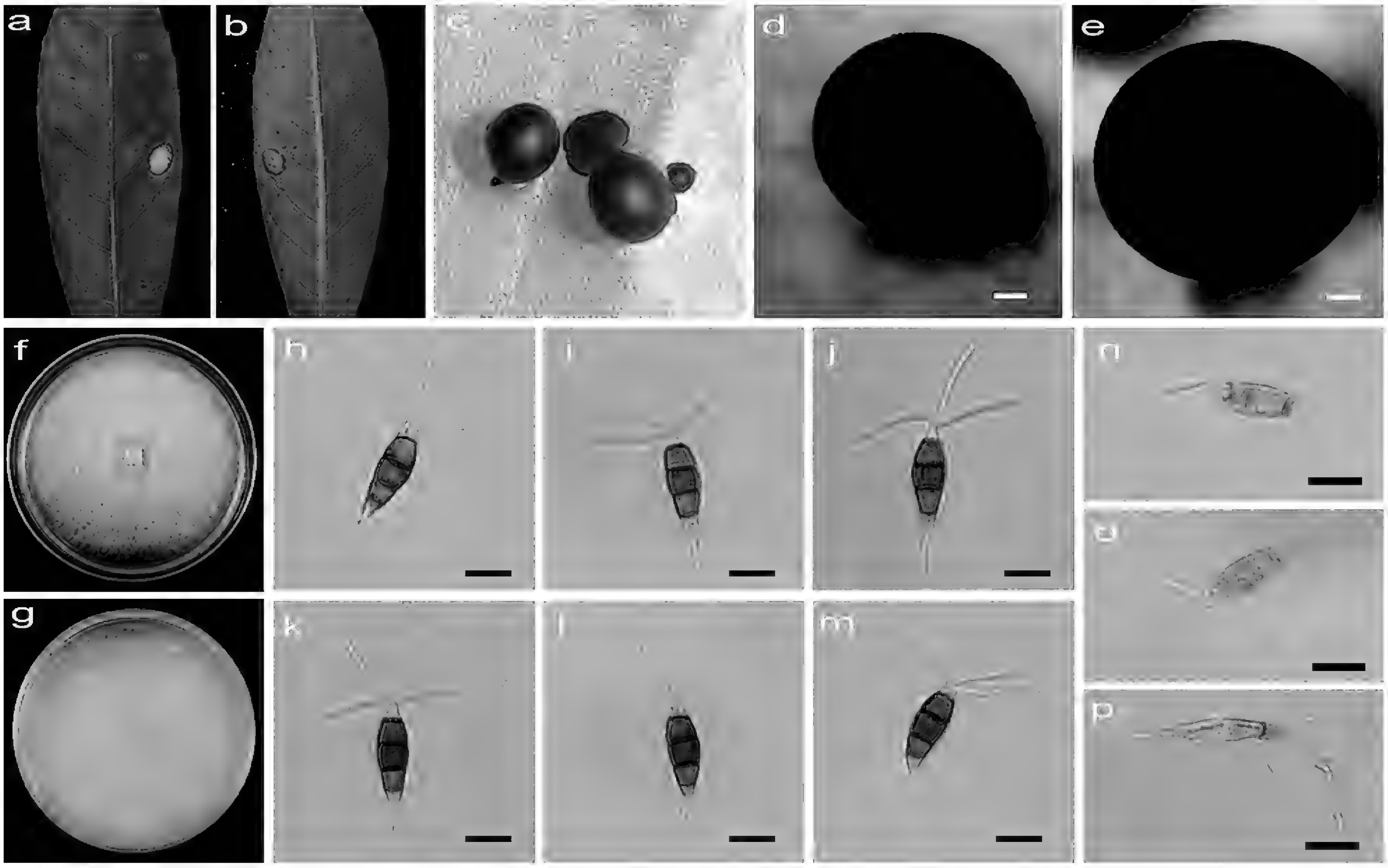

Figure 5. *Pestalotiopsis eriobotryae* (HJAUP C1742.221, ex-type) **a, b** leaf of host plant (front and reverse) **c–e** conidiomata **f, g** culture on PDA (front and reverse) **h–m** conidia **n–p** immature conidia. Scale bars: 200 μm (**d, e**); 10 μm (**h–p**).

Conidiomata acervular, globose, 839–2203 μm diam., solitary or aggregated in clusters, black. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform. Conidia fusiform, straight or slightly curved, 18.3–29.2 × 6.5–9 μm ($\bar{x}$ = 23.7 × 7.7 μm, n = 50), 4-septate, slightly constricted at the septa, basal cell conical, 2.8–5.3 μm ($\bar{x}$ = 4 μm), pale brown to subhyaline, smooth, thin-walled, with a single filiform appendage, unbranched, 4.1–11.5 μm ($\bar{x}$ = 7.1 μm) long; three median cells doliiform to cylindrical, smooth, 12.1–18.6 μm ($\bar{x}$ = 15.4 μm), concolorous or sometimes darker at the central cell or the two upper cells, somewhat constricted at the septa, second cell from the base pale brown, 3.4–6.9 μm ($\bar{x}$ = 5 μm) long, third cell medium to dark brown, 3.7–6.2 μm ($\bar{x}$ = 5.1 μm) long, fourth cell pale to medium brown, 4.4–6.5 μm ($\bar{x}$ = 5.4 μm) long; apical cell conical, hyaline, smooth, thin-walled, 3.4–5.3 μm ($\bar{x}$ = 4.2 μm) long, with 3–4 (mostly 3) filiform appendages, arising from the apex of the apical cell each at a different point, unbranched, 14.5–29.2 μm ($\bar{x}$ = 18.9 μm) long. Sexual morph not observed.

Culture characteristics. Colonies on PDA grow fast, filamentous to circular, reaching 81–85 mm diam. after 5 days at 25 °C in darkness, white to buff, with flocculent mycelium and entire edge, forming black conidiomata, and reverse pale orange.

Additional specimen examined. China • Jiangxi Province, Yingtan City, Guixi County, Shangqing Town, Longhu Mountain National Forest Park, 3 November 2022, X.X. Luo. On diseased leaves of *Eriobotrya japonica*, paratype HJAUP M1742.222, living culture HJAUP C1742.222.

Note. Two strains (HJAUP C1742.221 and HJAUP C1742.222) of *Pestalotiopsis eriobotryae* isolated from leaf spots of *Eriobotrya japonica* formed

a well-supported clade phylogenetically close to *P. doitungensis* (MFLUCC 14–0115) with 99% ML/0.95 BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1742.221 is closely related to *P. doitungensis* (MFLUCC 14–0115) and comparisons of their nucleotides showed 17 bp differences (2%, including three gaps) nucleotide differences in three loci. Moreover, *P. eriobotryae* is morphologically distinguished from *P. doitungensis* X.Y. Ma, K.D. Hyde & J.C. Kang in its wider conidia (6.5–9.0 µm vs. 5.5–6.5 µm) with more and longer apical filiform appendages (3–4 vs. 2–3, 14.5–29.2 µm vs. 4–12 µm) (Ma et al. 2019).

Pestalotiopsis gardeniae **X.X. Luo & Jian Ma, sp. nov.**
Index Fungorum: IF902323
Fig. 6

Type. China • Jiangxi Province, Yingtan City, Guixi County, Shangqing Town, Longhu Mountain National Forest Park, on diseased leaves of *Gardenia jasminoides*, 23 June 2022, X.X. Luo (holotype HJAUP M1729.221; ex-type living culture HJAUP C1729.221).

Description. Regular leaf spots, grey white in center, and pale brown at margin with yellowish halo. Asexual morph on PDA: Conidiomata acervular, globose or subglobular, 763–955 µm diam., solitary or aggregated, black. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform. Conidia fusiform, straight or slightly curved, 17.4–25.4 × 5.3–6.7 µm ($\bar{x}$ = 21.9 × 6 µm, n = 50), 4-septate, slightly constricted at the septa; basal cell conical, 3.4–6.4 µm ($\bar{x}$ = 5.1 µm), pale brown to subhyaline, smooth, thin-walled, with a single filiform appendage, unbranched, 2.9–4.7 µm ($\bar{x}$ = 3.9 µm) long; three median cells doliiform to cylindrical, 11–14.7 µm ($\bar{x}$ = 13.2 µm), concolorous or sometimes darker at the central cell or the two upper cells, somewhat constricted at the septa, second cell from the base pale brown, 3.4–5.1 ($\bar{x}$ = 4.3 µm) µm long, third cell medium to dark brown, 3.7–5.3 µm ($\bar{x}$ = 4.4 µm) long, fourth cell pale to medium brown, 3.7–5.4 µm ($\bar{x}$ = 4.5 µm) long; apical cell conical to acute, hyaline, smooth, thin-walled, 2.9–4.3 µm ($\bar{x}$ = 3.6 µm) long, with 2–3 (mostly 3) filiform appendages, arising from the apical crest, unbranched, 10–20.6 µm ($\bar{x}$ = 14.4 µm) long. Sexual morph not observed.

Culture characteristics. Colonies on PDA grow fast, filamentous to circular, reaching 70–75 mm diam. after 5 days at 25 °C in darkness, white, with flocculent aerial mycelium and entire edge, forming black conidiomata, and reverse pale orange.

Additional specimen examined. China • Jiangxi Province, Yingtan City, Guixi County, Shangqing Town, Longhu Mountain National Forest Park, 23 June 2022, X.X. Luo. On diseased leaves of *Gardenia jasminoides*, paratype HJAUP M1729.222, living culture HJAUP C1729.222; on diseased leaves of *Gardenia jasminoides*, paratype HJAUP M1729.223, living culture HJAUP C1729.223.

Note. Three strains (HJAUP C1729.221, HJAUP C1729.222 and HJAUP C1729.223) of *Pestalotiopsis gardeniae* isolated from leaf spots of *Gardenia jasminoides* formed a distinct clade sister to *P. sichuanensis* (SA3A21) with 100% ML/1.00 BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1729.221 is closely related to *P. sichuanensis* (SA3A21) and comparisons of their nucleo-

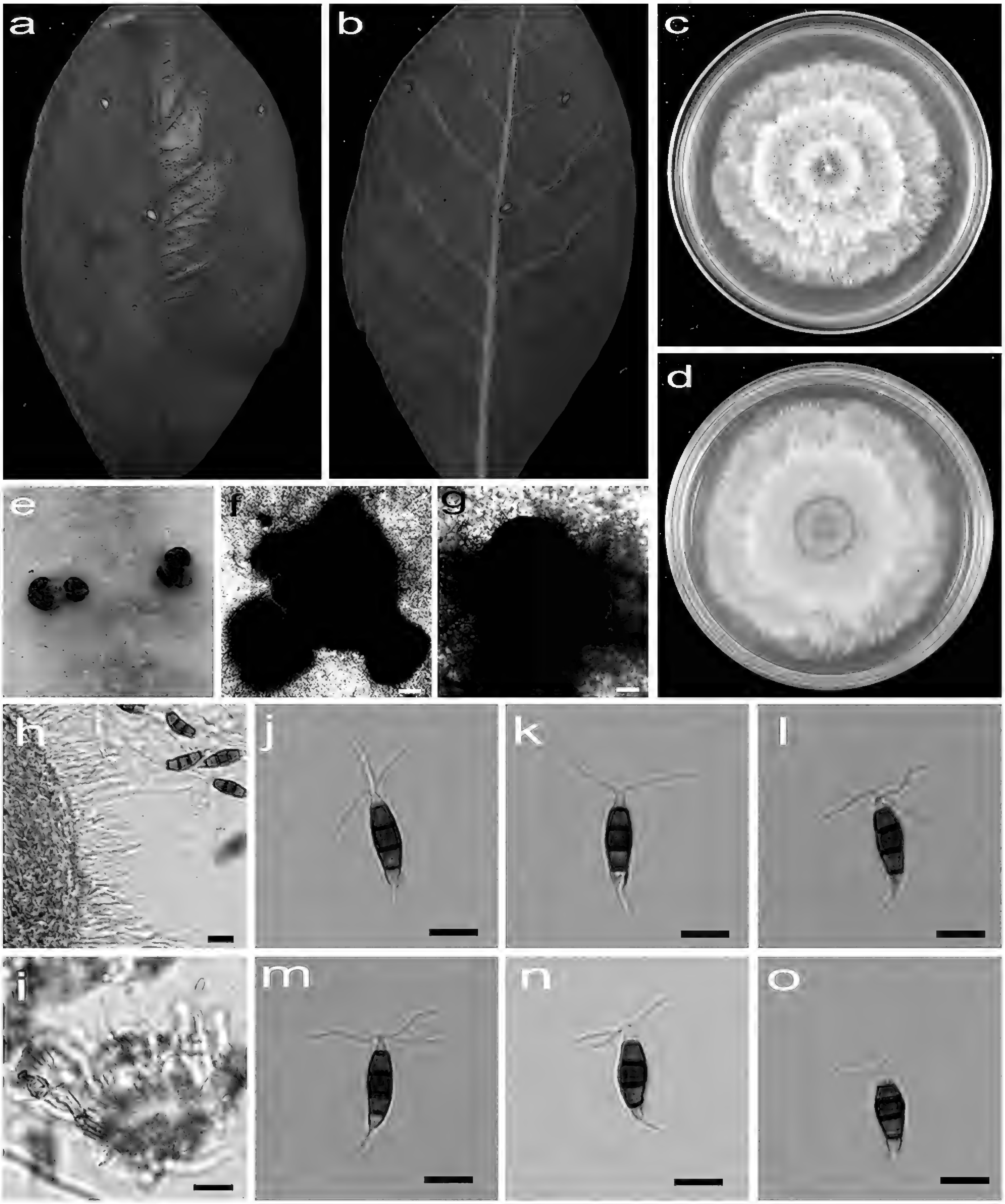

Figure 6. *Pestalotiopsis gardeniae* (HJAUP C1729.221, ex-type) **a, b** leaf of host plant (front and reverse) **c, d** culture on PDA (front and reverse) **e–g** conidiomata **h, i** conidiogenous cells and conidia **j–o** conidia. Scale bars: 200 μm (**f, g**); 10 μm (**h–o**).

tides showed 3 bp differences (1%, including zero gap) nucleotide differences in three loci. Moreover, *P. gardeniae* is morphologically distinguished from *P. sichuanensis* Y.C. Wang, X.C. Wang & Y.J. Yang in its larger conidia (17.4–25.4 × 5.3–6.7 μm vs. 8.6–12.5 × 2.6–3.7 μm) with longer apical filiform appendages (10–20.6 μm vs. 2.6–9.2 μm) (Wang et al. 2019).

Pestalotiopsis hederae **X.X. Luo & Jian Ma, sp. nov.**
Index Fungorum: IF902324
Fig. 7

Type. China • Yunnan Province, Jinghong City, Menghan Town, Xishuangbanna Dai Nationality Garden; on diseased leaves of *Hedera helix*; 22 June 2022, X.X. Luo (holotype HJAUP M1638.221; ex-type living culture HJAUP C1638.221).

Etymology. Referring to the host genus, *Hedera* from which it was collected.

Description. Regular leaf spots, grey-brown in the center and darkening to black brown at the margins. Asexual morph on PDA: Conidiomata acervular, globose, 660–1570 μm diam., solitary or aggregated in clusters, black. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform. Conidia fusiform, straight or slightly curved, 15.8–22.4 × 4.9–6.3 μm ($\bar{x}$ = 18.0 × 5.7 μm, n = 50), 4-septate, slightly constricted at the septa, basal cell conical, 3.1–5.3 μm ($\bar{x}$ = 4 μm), hyaline or sometimes pale brown, smooth, thin-walled, with a single filiform appendage, unbranched, 3.4–5.9 μm ($\bar{x}$ = 4.8 μm) long; three median cells doliiform to cylindrical, smooth, thick-walled, 11.1–15.5 μm ($\bar{x}$ = 13.6 μm), pale brown to brown, concolorous, somewhat constricted at the septa, second cell from the base 3.7–5.3 μm ($\bar{x}$ = 4.7 μm) long, third cell 4.3–5.6 μm ($\bar{x}$ = 4.9 μm) long, fourth cell 4.2–5.6 μm ($\bar{x}$ = 5 μm) long; apical cell

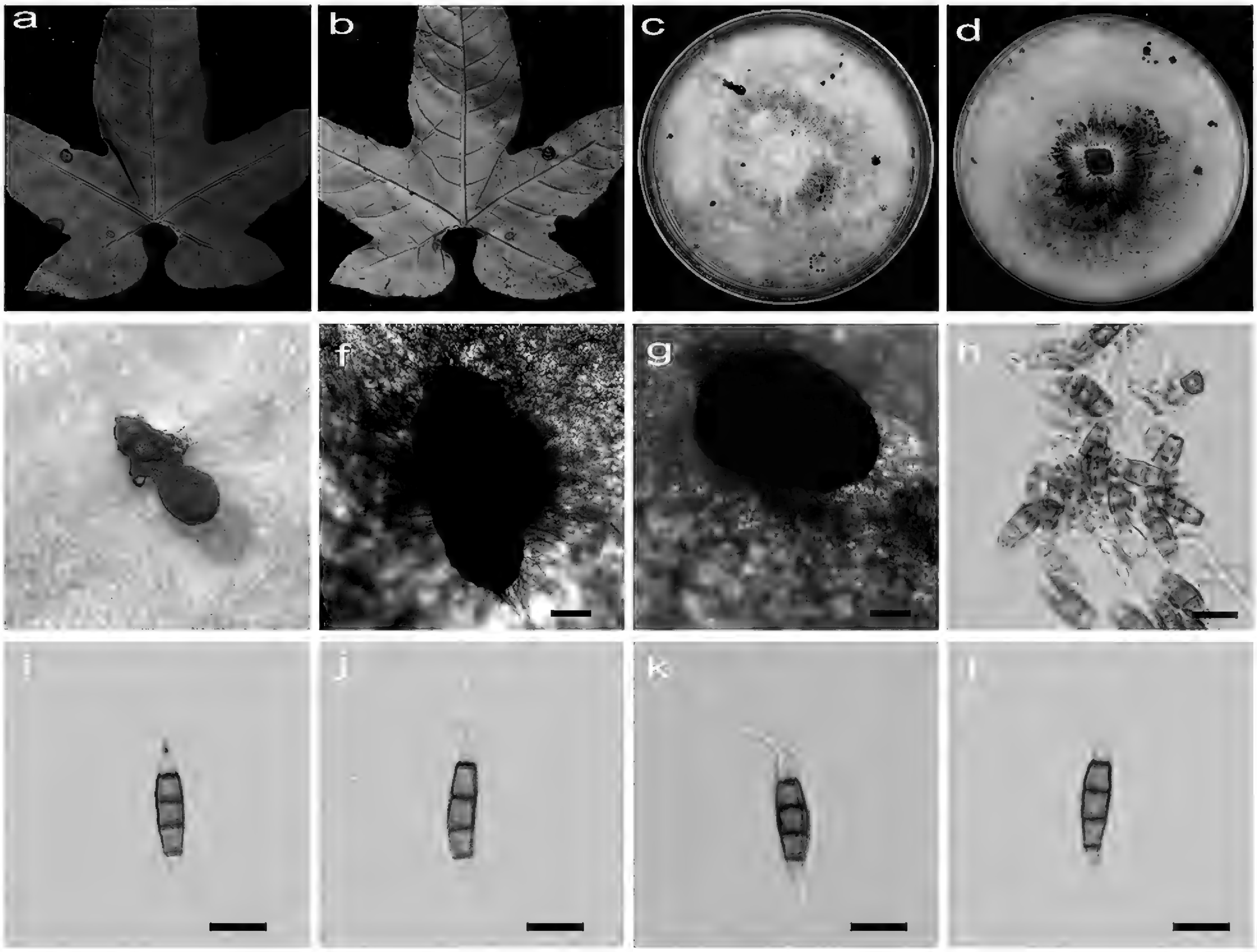

Figure 7. *Pestalotiopsis hederae* (HJAUP C1638.221, ex-type) **a, b** leaf of host plant (front and reverse) **c, d** culture on PDA (front and reverse) **e–g** conidiomata **h** conidiogenous cells and conidia **i–k** conidia. Scale bars: 200 μm (**f, g**); 10 μm (**h–l**).

conical to acute, hyaline, smooth, thin-walled, 3.3–5 μm ($\bar{x}$ = 4.1 μm) long, with 2(–3) filiform appendages, arising from the apex of the apical cell each at a different point, unbranched, 10.8–19.6 μm ($\bar{x}$ = 15.5 μm) long. Sexual morph not observed.

Culture characteristics. Colonies on PDA grow fast, filamentous to circular, growing all over the Petri dish at 25 °C in darkness, regular edge, white, sparse aerial mycelium on the surface, forming black conidiomata with black conidial masses, and reverse pale orange or white at the margin, dark brown at the center.

Additional specimen examined. China • Yunnan Province, Jinghong City, Menghan Town, Xishuangbanna Dai Nationality Garden, 22 June 2022, X.X. Luo. On diseased leaves of *Hedera helix*, paratype HJAUP M1638.222, living culture HJAUP C1638.222.

Note. Two strains (HJAUP C1638.221 and HJAUP C1638.222) of *Pestalotiopsis hederae* isolated from leaf spots of *Hedera helix* formed a distinct clade sister to *P. hydei* (MFLUCC 20–0135) with 94% ML/0.95 BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1638.221 is closely related to *P. hydei* (MFLUCC 20–0135) and comparisons of their nucleotides showed 10 bp differences (1%, including two gaps) nucleotide differences in three loci, respectively. Moreover, *P. hederae* is morphologically distinguished from *P. hydei* Huanraluek & Jayaward., which has longer conidia (18–35 μm vs. 15.8–22.4 μm) with minutely verruculose three median cells and shorter apical appendages (3–12 μm vs. 10.8–19.6 μm) (Huanaluek et al. 2021).

Pestalotiopsis machiliana **X.X. Luo and Jian Ma, sp. nov.**

Index Fungorum: IF902325

Fig. 8

Type. China • Jiangxi Province, Jingdezhen City, Changjiang District, Jingdezhen Botanical Garden; on diseased leaves of *Machilus pauhoi*; 3 November 2022; X.X. Luo (holotype HJAUP M1790.221; ex-type living culture HJAUP C1790.221).

Etymology. Referring to the host genus, *Machilus* from which it was collected.

Description. Regular leaf spots, wheat in the center, a black stripe ring in the middle and dark brown at the margin. Asexual morph on PDA: Conidiomata acervular, globose, 646–1584 μm diam., solitary or aggregated in clusters, black. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform. Conidia fusiform, straight or slightly curved, 18.6–27.2 × 5.6–7.4 μm ($\bar{x}$ = 22.5 × 6.5 μm, n = 50), 4-septate, slightly constricted at the septa; basal cell conical, 3–5.2 μm ($\bar{x}$ = 3.9 μm), hyaline or sometimes pale brown, smooth, thin-walled, with a single filiform appendage, unbranched, 4.5–10.2 μm ($\bar{x}$ = 8.1 μm) long; three median cells doliiform to cylindrical, smooth, 12.5–17.3 μm ($\bar{x}$ = 14.7 μm), concolorous, brown, somewhat constricted at the septa, second cell from the base 3.6–6.7 μm ($\bar{x}$ = 5.0 μm) long, third cell 3.8–5.5 μm ($\bar{x}$ = 4.6 μm) long, fourth cell 4.1–6.4 μm ($\bar{x}$ = 4.9 μm) long; apical cell conical to acute, hyaline, smooth, thin-walled, 3–4.8 μm ($\bar{x}$ = 3.9 μm) long, with 2–3 filiform appendages, arising from the apex of the apical cell each at a different point, unbranched, 12.9–22.5 μm ($\bar{x}$ = 14.7 μm) long. Sexual morph not observed.

Culture characteristics. Colonies on PDA grow fast, reaching 47–53 mm diam. after 5 days at 25 °C in darkness, white, with flocculent mycelium and entire edge, forming black conidiomata, and reverse buff.

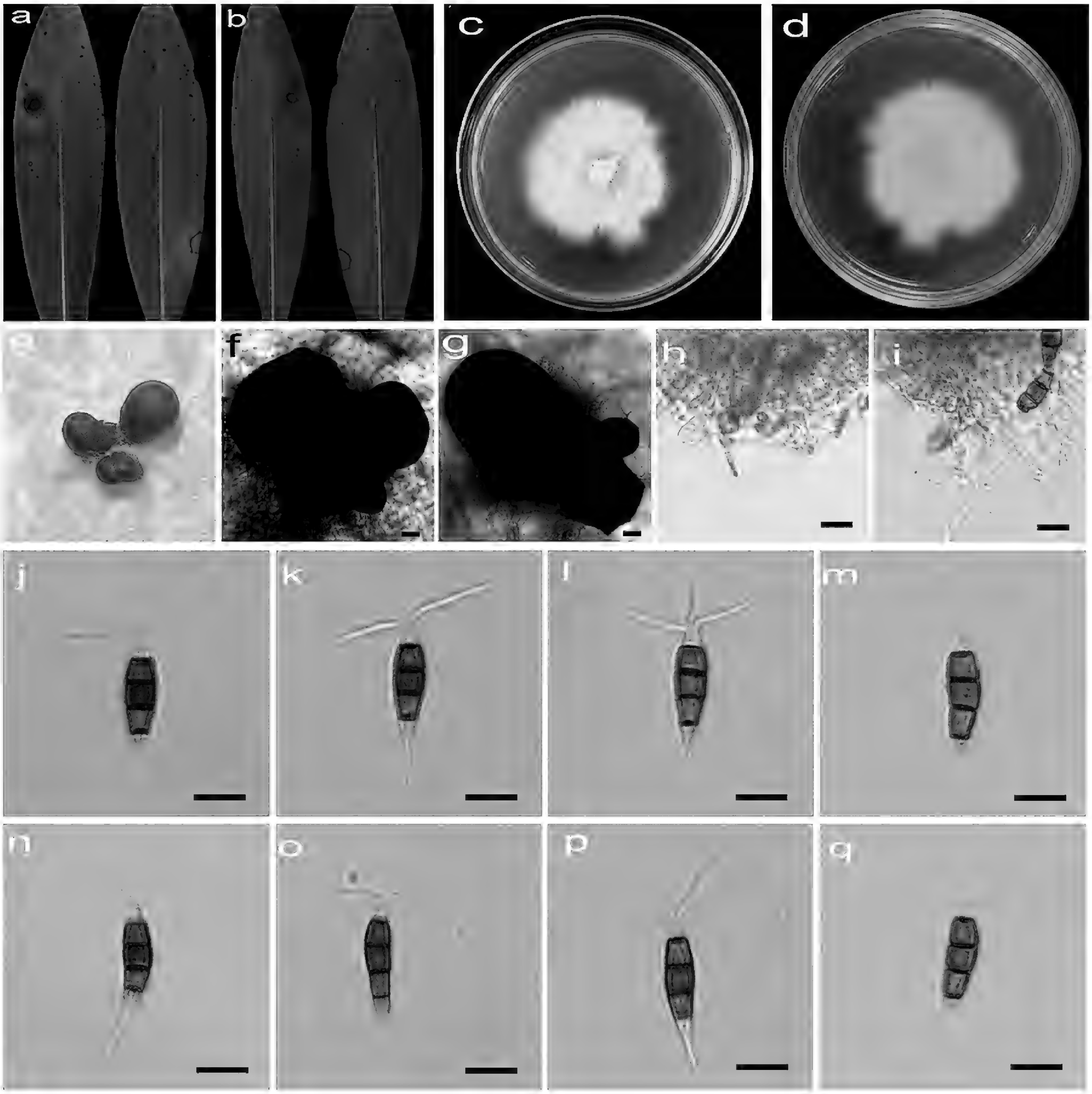

Figure 8. *Pestalotiopsis machiliana* (HJAUP C1790.221, ex-type) **a, b** leaf of host plant (front and reverse) **c, d** culture on PDA (front and reverse) **e–g** conidiomata **h, i** conidiogenous cells and conidia **j–q** conidia. Scale bars: 200 μm (**f, g**); 10 μm (**h–q**).

Additional specimens examined. China, Jiangxi Province, Jingdezhen City • Changjiang District, Jingdezhen Botanical Garden, 3 November 2022, X.X. Luo. On diseased leaves of *Machilus pauhoi*, paratype HJAUP M1790.222, living culture HJAUP C1790.222 • Fuliang County, Jingdezhen National Forest Park, 2 November 2022, X.X. Luo, on diseased leaves of *Rhododendron simsii*, paratype HJAUP M1704.221, living culture HJAUP C1704.221; on diseased leaves of *Rhododendron simsii*, paratype HJAUP M1704.222, living culture HJAUP C1704.222; on diseased leaves of *Rhododendron simsii*, paratype HJAUP M1704.223, living culture HJAUP C1704.223.

Note. Five strains (HJAUP C1790.221, HJAUP C1790.222, HJAUP C1704.221, HJAUP C1704.222 and HJAUP C1704.223) of *Pestalotiopsis machiliana* isolated from leaf spots of *Machilus pauhoi* clustered as a sister taxon to *P. chamaeropis* (CFCC 54977, CFCC 55023, CFCC 55019 and CFCC 55122) with 99% ML/0.97

BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1790.221 is closely related to *P. chamaeropis* (CBS 186.71) and comparisons of their nucleotides showed 8 bp differences (1%, including one gap) nucleotide differences in three loci. Moreover, *P. machiliana* is morphologically distinguished from *P. chamaeropis* Maharachch., K.D. Hyde & Crous, which has minutely verruculose, wider conidia (7–9 µm vs. 5.6–7.4 µm) with longer basal cell (5–6.5 µm vs. 3–5.2 µm) and apical cell (4–6 µm vs. 3–4.8 µm) (Maharachchikumbura et al. 2014).

Pestalotiopsis mangifericola X.X. Luo & Jian Ma, sp. nov.
Index Fungorum: IF902326
Fig. 9

Type. CHINA • Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun Town, Tropical Botanical Garden, on diseased leaves of *Mangifera indica*, 23 June 2022, X.X. Luo (holotype HJAUP M1639.221; ex-type living culture HJAUP C1639.221).

Etymology. Referring to the host genus, *Mangifera* from which it was collected.

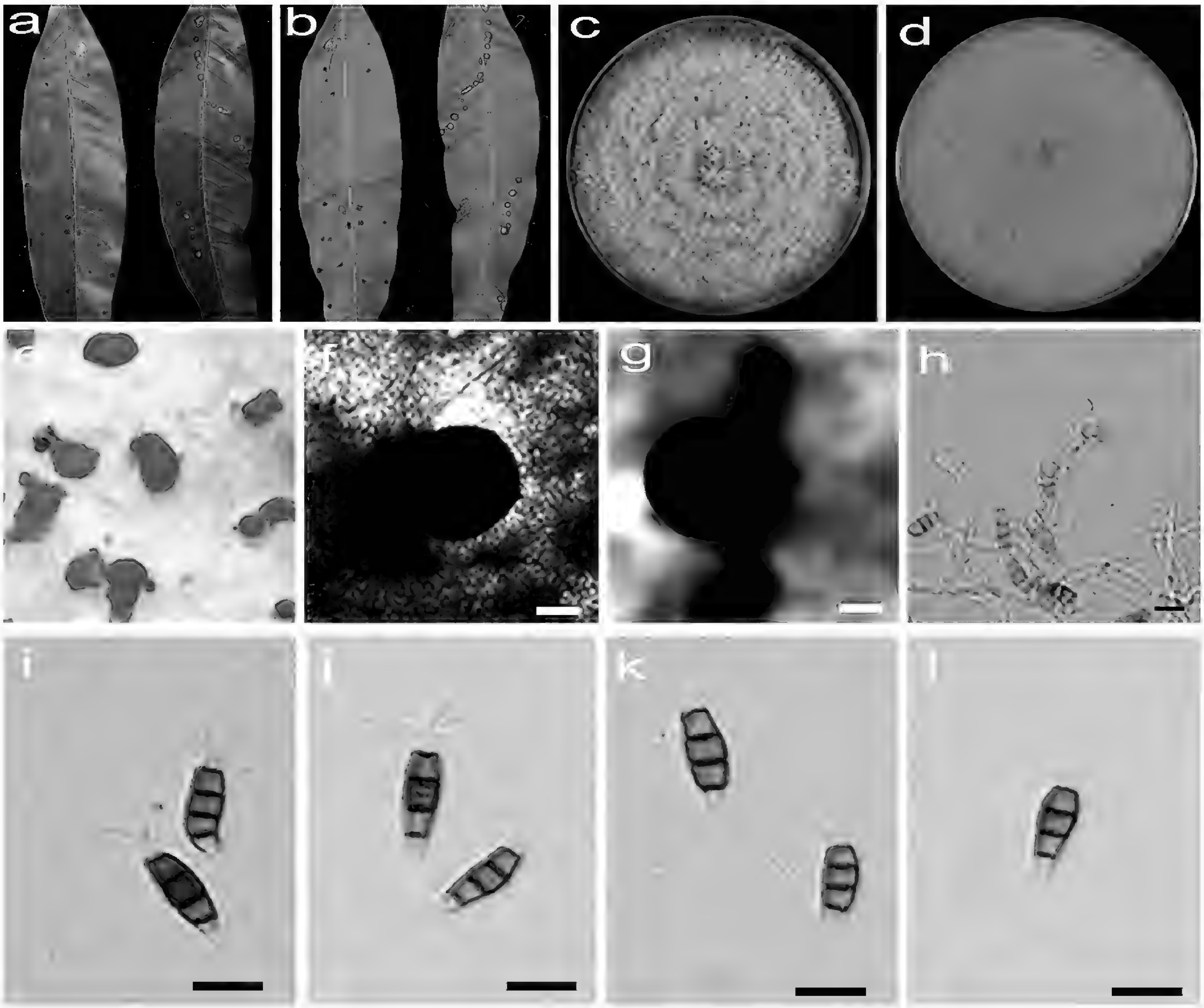

Figure 9. *Pestalotiopsis mangifericola* (HJAUP C1639.221, ex-type) **a, b** leaf of host plant (front and reverse) **c, d** culture on PDA (front and reverse) **e–g** conidiomata **h** conidiogenous cells and conidia **i–l** conidia. Scale bars: 200 µm (**f, g**); 10 µm (**h–l**).

Description. Regular leaf spots, initially brown with a yellowish halo around the edges, later yellowish-white center with black edges. Asexual morph on PDA: Conidiomata acervular, subglobular, 426–786 µm diam, solitary or aggregated in clusters, black. Conidiophores indistinct and reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform. Conidia fusiform, straight or slightly curved, 13.5–18 × 4.7–6 µm ($\bar{x}$ = 15.2 × 5.4 µm, n = 50), 4-septate, slightly constricted at the septa; basal cell conical, 2.9–4.4 µm ($\bar{x}$ = 3.6 µm), hyaline or sometimes pale brown, smooth, thin-walled, with a single filiform appendage, unbranched, 3.1–5.5 µm ($\bar{x}$ = 4.2µm) long; three median cells doliiform to cylindrical, smooth, 10.8–12.3 µm ($\bar{x}$ = 11.5 µm), concolorous or sometimes darker at the central cell or the two upper cells, somewhat constricted at the septa, second cell from the base pale brown, 3.3–4.6 µm ($\bar{x}$ = 3.9 µm) long, third cell pale brown to brown, 3.6–4.5 µm ($\bar{x}$ = 3.9 µm) long, fourth cell pale to medium brown, 3.5–5.1 µm ($\bar{x}$ = 4.2 µm) long; apical cell conical to acute, hyaline, smooth, thin-walled, 2.5–4 µm ($\bar{x}$ = 3.1 µm) long, with 2–3 filiform appendages, arising from the apical crest, unbranched, 7.2–11.6 µm ($\bar{x}$ = 9.8 µm) long. Sexual morph not observed.

Culture characteristics. Colonies on PDA grow fast, filamentous to circular, growing all over the Petri dish (d = 8.5 cm) after 2 weeks at 25 °C in darkness, white, with flocculent aerial mycelium and entire edge, forming black conidiomata, and reverse pale orange.

Additional specimen examined. China • Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun Town, Tropical Botanical Garden, 23 June 2022, X.X. Luo. On diseased leaves of *Mangifera indica*, paratype HJAUP M1639.222, living culture HJAUP C1639.222.

Note. Two strains (HJAUP C1639.221 and HJAUP C1639.222) of *Pestalotiopsis mangifericola* isolated from leaf spots of *Mangifera indica* formed a distinct clade sister to *P. adusta* (MFLUCC 10–146 and ICMP 6088) with 100% ML/0.90 BI bootstrap support (Fig. 1). The ex-type strain HJAUP C1639.221 is closely related to *P. adusta* (ICMP 6088) and comparisons of their nucleotides showed 4 bp differences (1%, including one gap) nucleotide differences in three loci. Moreover, *P. mangifericola* is morphologically distinguished from *P. adusta* (Ellis & Everh.) Steyaert in its smaller conidia (13.5–18 × 4.7–6 µm vs. 16–22 × 5–7 µm) with shorter three median cells (10.8–12.3 µm vs. 12–15 µm) (Steyaert 1953; Maharachchikumbura et al. 2012).

Discussion

The establishment of *Pestalotiopsis* was based on morphological studies. Members in the genus mainly occur in the asexual morph, and only 12 species have been linked with the sexual morphs (Maharachchikumbura et al. 2011). The generic concept of *Pestalotiopsis* is based on the characteristics of asexual morph and is mainly characterized by fusiform conidia and three pigmented median cells, each consisting of a hyaline basal cell and a hyaline apical cell with one or more simple or branched appendages (Steyaert 1949; Maharachchikumbura et al. 2014). These characters separate *Pestalotiopsis* from *Pestalotia* De Not. (with 6-celled conidia) and *Truncatella* Steyaert (with 4-celled conidia). Subsequently, Maharachchikumbura et al. (2014) revisited the genus *Pestalotiopsis* based on molecular evidence and the differences in the median cells of the conidia and proposed two segregated genera including

Neopestalotiopsis and *Pseudopestalotiopsis*. Senanayake et al. (2015) treated *Pestalotiopsis*, *Pseudopestalotiopsis*, *Neopestalotiopsis* and other four genera in a new family, Pestalotiopsidaceae Maharachch. & K.D. Hyde, based on morphological similarities and sequence analysis.

To date, about 437 epithets for *Pestalotiopsis* have been listed in Index Fungorum (Index Fungorum 2024), but many species were introduced only based on morphological studies, and the excessive overlap of conidial features makes it difficult to identify *Pestalotiopsis* species only by morphology. Thus, there is presently a strong tendency to evaluate or clarify the taxonomic placements and phylogenetic relationships of *Pestalotiopsis* species by molecular methods. Maharachchikumbura et al. (2014) analyzed ten gene regions to resolve the bound species in *Neopestalotiopsis* and *Pestalotiopsis*, and finally screened three most applicable regions (ITS, *tef1-α*, and *tub2*). Since then, the number of *Pestalotiopsis* species is constantly being excavated and steadily increasing, and all described *Pestalotiopsis* species were identified based on the combined analyses of these three loci except for *P. sequoia*, *P. bulbophylli* and *P. chiaroscuro* using LSU, ITS, *tef1-α* and *tub2* (Hyde et al. 2016; Wang et al. 2017; Crous et al. 2022). Our BLASTn analyses of these sequences showed a high similarity in some *Pestalotiopsis* species, such as ITS, *tef1-α* and *tub2* of *P. ficicrescens* (MZ477311, MZ868328 and MZ868301) (Hyde et al. 2023) were 99.62, 99.79 and 98.56% similar to *P. biciliata* (KM199308, KM199505 and KM199399) (Maharachchikumbura et al. 2014); *P. taxicola* (OQ626673, OQ714338 and OQ714333) (Wang et al. 2024) were 100%, 99.25% and 100% similar to *P. unicolor* (JX398998, JX399063 and JX399029) (Maharachchikumbura et al. 2012); *P. linguae* (OP094104, OP186110 and OP186108) (Li et al. 2023) were 99.64, 98.74 and 98.26 similar to *P. parva* (KM199313, KM199509 and KM199405) (Maharachchikumbura et al. 2014), but the phylogenetic analyses conducted based on combined ITS, *tef1-α* and *tub2* sequence data showed more powerful resolution in delineating *Pestalotiopsis* species and higher bootstrap support values for most clades. Based on previous studies, we also conducted phylogenetic analyses using ITS, *tef1-α* and *tub2* sequences, and our newly obtained 24 strains nested within the genus *Pestalotiopsis* formed distinct clades with good support value, and can be recognized as eight new phylogenetic species.

Pestalotiopsis species are known worldwide as plant pathogens, endophytes, or saprophytes, and are widely distributed in tropical and temperate regions (Maharachchikumbura et al. 2014; Li et al. 2024; Zhao et al. 2024). In recent years, studies conducted on the alpha-taxonomy of *Pestalotiopsis* are mainly focused on the exploration of the hidden species diversity (Index Fungorum 2024). The leaves with typical spots diseased by *Pestalotiopsis* fungi are usually collected to obtain fungal isolates, and the strains are identified based on morphological and phylogenetic approaches, but little attention has been accorded to their pathogenicity. In our study, the survey of microfungi associated with plant diseased leaves from terrestrial habitat in Jiangxi and Yunnan provinces, China reveal eight new species, namely *P. alpinicola*, *P. camelliicola*, *P. cyclosora*, *P. eriobotryae*, *P. gardeniae*, *P. hederae*, *P. machiliana* and *P. mangifericola*. To our knowledge, *P. alpinicola*, *P. cyclosora* and *P. machiliana* are the first report that associated with the hosts *Alpinia zerumbet*, *Cyclosorus interruptus*, *Machilus pauhoi* and *Microlepia marginata*, which will broaden the host range of *Pestalotiopsis* species, and provide an important contribution to

the field of plant pathology and fungal taxonomy. With the ongoing addition of *Pestalotiopsis* species, we believe that a comprehensive study of the genus will reveal more hidden *Pestalotiopsis* species from terrestrial plants.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Sampling: X.X.L.; Fungal isolation: M.G.L.; Microscopy: X.X.L.; Description and phylogenetic analyses: X.X.L. and K.Z.; Writing – original draft preparation: X.X.L.; Writing – review and editing, R.F.C., Z.H.X. and J.M. All authors read and approved the final manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Barr ME (1975) *Pestalosphaeria*, a new genus in the Amphisphaeriaceae. Mycologia 67(1): 187–194. https://doi.org/10.1080/00275514.1975.12019740

Barr ME (1990) Prodromus to nonlichenized, pyrenomycetous members of class Hymenoascomycetes. Mycotaxon 39: 43–184.

Bate-Smith EC, Metcalfe CR (1957) Leuco–anthocyanins. 3. The nature and systematic distribution of tannin in dicotyledonous plants. Journal of the Linnean Society of London, Botany 55(362): 669–705. https://doi.org/10.1111/j.1095-8339.1957.tb00030.x

Bhunjun CS, Niskanen T, Suwannarach N, Wannathes N, Chen YJ, McKenzie EHC, Maharachchikumbura SSN, Buyck B, Zhao CL, Fan YG, Zhang JY, Dissanayake AJ, Marasinghe DS, Jayawardena RS, Kumla J, Padamsee M, Chen YY, Liimatainen K, Ammirati JF, Phukhamsakda C, Liu JK, Phonrob W, Randrianjohany É, Hongsanan S, Cheewangkoon R, Bundhun D, Khuna S, Yu WJ, Deng LS, Lu YZ, Hyde KD, Lumyong S (2022) The numbers of fungi: Are the most speciose genera truly diverse? Fungal Diversity 114(1): 387–462. https://doi.org/10.1007/s13225-022-00501-4

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/00275514.1999.12061051

Crous PW, Boers J, Holdom D, Osieck ER, Steinrucken TV, Tan YP, Vitelli JS, Shivas RG, Barrett M, Boxshall AG, Broadbridge J, Larsson E, Lebel T, Pinruan U, Sommai S, Alvarado P, Bonito G, Decock CA, De la Peña-Lastra S, Delgado G, Houbraken J, Maciá-Vicente JG, Raja HA, Rigueiro-Rodríguez A, Rodríguez A, Wingfield MJ, Adams SJ, Akulov A, Al-Hidmi T, Antonín V, Arauzo S, Arenas F, Armada F, Aylward J, Bellanger JM, Berraf-Tebbal A, Bidaud A, Boccardo F, Cabero J, Calledda F, Corriol G, Crane JL, Dearnaley JDW, Dima B, Dovana F, Eichmeier A, Esteve-Raventós F, Fine M, Ganzert L, García D, Torres-Garcia D, Gené J, Gutiérrez A, Iglesias P, Istel Ł, Jangsantear P, Jansen GM, Jeppson M, Karun NC, Karich A, Khamsuntorn P, Kokkonen K, Kolařík M, Kubátová A, Labuda R, Lagashetti AC, Lifshitz N, Linde C, Loizides M, Luangsa-Ard JJ, Lueangjaroenkit P, Mahadevakumar S, Mahamedi AE, Malloch DW, Marincowitz S, Mateos A, Moreau PA, Miller AN, Molia A, Morte A, Navarro-Ródenas A, Nebesářová J, Nigrone E, Nuthan BR, Oberlies NH, Pepori AL, Rämä T, Rapley D, Reschke K, Robicheau BM, Roets F, Roux J, Saavedra M, Sakolrak B, Santini A, Ševčíková H, Singh PN, Singh SK, Somrithipol S, Spetik M, Sridhar KR, Starink-Willemse M, Taylor VA, van Iperen AL, Vauras J, Walker AK, Wingfield BD, Yarden O, Cooke AW, Manners AG, Pegg KG, Groenewald JZ (2022) Fungal Planet description sheets: 1383–1435. Persoonia 48(1): 261–371. https://doi.org/10.3767/persoonia.2022.48.08

Gao YH, Sun W, Su YY, Cai L (2014) Three new species of *Phomopsis* in Gutianshan nature reserve in China. Mycological Progress 13(1): 111–121. https://doi.org/10.1007/s11557-013-0898-2

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from Filamentous Ascomycetes. Applied and Environmental Microbiology 61(4): 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Griffiths DA, Swart HJ (1974a) Conidial structure in two species of *Pestalotiopsis*. Transactions of the British Mycological Society 62(2): 295–304. https://doi.org/10.1016/S0007-1536(74)80038-0

Griffiths DA, Swart HJ (1974b) Conidial structure in *Pestalotia pezizoides*. Transactions of the British Mycological Society 63(1): 169–173. https://doi.org/10.1016/S0007-1536(74)80149-X

Guba EF (1961) Monograph of *Monochaetia* and *Pestalotia*. Harvard University Press, Cambridge.

Hsu SY, Xu YC, Lin YC, Chuang WY, Lin SR, Stadler M, Tangthirasunun N, Cheewangkoon R, AL-Shwaiman HA, Elgorban AM, Ariyawansa HA (2024) Hidden diversity of *Pestalotiopsis* and *Neopestalotiopsis* (Amphisphaeriales, Sporocadaceae) species allied with the stromata of entomopathogenic fungi in Taiwan. MycoKeys 101: 275–312. https://doi.org/10.3897/mycokeys.101.113090

Huanaluek N, Jayawardena RS, Maharachchikumbura SS, Harishchandra DL (2021) Additions to pestalotioid fungi in Thailand: *Neopestalotiopsis hydeana* sp. nov. and *Pestalotiopsis hydei* sp. nov. Phytotaxa 479(1): 23–43. https://doi.org/10.11646/phytotaxa.479.1.2

Hyde KD, Hongsanan S, Jeewon R, Bhat DJ, McKenzie EH, Jones EB, Phookamsak R, Ariyawansa HA, Boonmee S, Zhao Q, Abdel-Aziz FA, Abdel-Wahab MA, Banmai S, Chomnunti P, Cui B, Daranagama DA, Das K, Dayarathne MC, de Silva N, Dissanayake AJ, Doilom M, Ekanayaka AH, Gibertoni TB, Góes-Neto A, Huang S, Jayasiri SC, Jayawardena RS, Konta S, Lee HB, Li W, Lin C, Liu J, Lu Y, Luo Z, Manawasinghe IS,

Manimohan P, Mapook A, Niskanen T, Norphanphoun C, Papizadeh M, Perera RH, Phukhamsakda C, Richter C, et al. (2016) Fungal diversity notes 367–490: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80: 1–270. https://doi.org/10.1007/s13225-016-0373-x

Hyde KD, Suwannarach N, Jayawardena RS, Manawasinghe IS, Liao CF, Doilom M, Cai L, Zhao P, Buyck B, Phukhamsakda C, Su WX, Fu YP, Li Y, Zhao RL, He MQ, Li JX, Tibpromma S, Lu L, Tang X, Kang JC, Ren GC, Gui H, Hofstetter V, Ryoo R, Antonín V, Hurdeal VG, Gentikaki E, Zhang JY, Lu YZ, Senanayake IC, Yu FM, Zhao Q, Bao DF (2021) Mycosphere notes 325–344 – Novel species and records of fungal taxa from around the world. Mycosphere: Journal of Fungal Biology 12(1): 1101–1156. https://doi.org/10.5943/mycosphere/12/1/14

Hyde KD, Norphanphoun C, Ma J, Yang HD, Zhang JY, Du TY, Gao Y, Gomes de Farias AR, He S, He YK, Li CJ, Li JY, Liu XF, Lu L, Su HL, Tang X, Tian XG, Wang SY, Wei DP, Xu RF, Xu RJ, Yang YY, Zhang F, Zhang Q, Bahkali AH, Boonmee S, Chethana KWT, Jayawardena RS, Lu YZ, Karunarathna SC, Tibpromma S, Wang Y, Zhao Q (2023) Mycosphere notes 387–412 – novel species of fungal taxa from around the world. Mycosphere: Journal of Fungal Biology 14(1): 663–744. https://doi.org/10.5943/mycosphere/14/1/8

Index Fungorum (2024) Index Fungorum. http://www.indexfungorum.org/Names/Names.asp [accessed on 6 september 2024]

Jiang N, Voglmayr H, Xue H, Piao CG, Li Y (2022) Morphology and phylogeny of *Pestalotiopsis* (Sporocadaceae, Amphisphaeriales) from Fagaceae Leaves in China. Microbiology Spectrum 10(6): e03272–e22. https://doi.org/10.1128/spectrum.03272-22

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Kang JC, Kong RYC, Hyde KD (1998) Studies on the Amphisphaeriales I. Amphisphaeriaceae (*sensu stricto*) and its phylogenetic relationships inferred from 5.8 S rDNA and ITS2 sequences. Fungal Diversity 1: 147–157.

Kang JC, Hyde KD, Kong RYC (1999) Studies on the Amphisphaeriales: The Amphisphaeriaceae (*sensu stricto*). Mycological Research 103(1): 53–64. https://doi.org/10.1017/S0953756298006650

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Li J, Xie J, Li XN, Zhou ZF, Liu FL, Chen YH (2017) Isolation, identification and antimicrobial activity of mycoparasites (*Pestalotiopsis*) from *Aecidium pourthiaea*. Shengwu Jishu Tongbao 33: 122. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2017.03.018

Li H, Manawasinghe IS, Zhang YX, Senanayake IC (2023) Taxonomic and hylogenic appraisal of *Pestalotiopsis linguae* sp. nov., and a new record of *P. nanjingensis* from *Pyrrosia lingua* (Polypodiaceae) in Southern China. Phytotaxa 587(3): 229–250. https://doi.org/10.11646/phytotaxa.587.3.3

Li H, Peng BY, Xie JY, Bai YQ, Li DW, Zhu LH (2024) *Pestalotiopsis jiangsuensis* sp. nov. causing needle blight on *Pinus massoniana* in China. Journal of Fungi (Basel, Switzerland) 10(3): 230. https://doi.org/10.3390/jof10030230

Lv C, Huang B, Qiao M, Wei J, Ding B (2011) Entomopathogenic fungi on *Hemiberlesia pitysophila*. PLoS One 6(8): e23649. https://doi.org/10.1371/journal.pone.0023649

Ma XY, Maharachchikumbura SSN, Chen BW, Hyde KD, McKenzie EHC, Chomnunti P, Kang JC (2019) Endophytic *pestalotiod* taxa in *Dendrobium* orchids. Phytotaxa 419(3): 268–286. https://doi.org/10.11646/phytotaxa.419.3.2

Maharachchikumbura SSN, Guo LD, Chukeatirote E, Bahkali AH, Hyde KD (2011) *Pestalotiopsis*-morphology, phylogeny, biochemistry and diversity. Fungal Diversity 50(1): 167–187. https://doi.org/10.1007/s13225-011-0125-x

Maharachchikumbura SSN, Guo LD, Cai L, Chukeatirote E, Wu WP, Sun X, Crous PW, Bhat DJ, McKenzie EHC, Bahkali AH, Hyde KD (2012) A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Diversity 56(1): 95–129. https://doi.org/10.1007/s13225-012-0198-1

Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW (2014) *Pestalotiopsis* revisited. Studies in Mycology 79(1): 121–186. https://doi.org/10.1016/j.simyco.2014.09.005

Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi.org/10.1093/molbev/mst024

Monden Y, Yamamoto S, Yamakawa R, Sunada A, Asari S, Makimura K, Inoue Y (2013) First case of fungal keratitis caused by *Pestalotiopsis clavispora*. Clinical Ophthalmology (Auckland, N.Z.) 7: 2261–2264. https://doi.org/10.2147/OPTH.S48732

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Schimann H, Bach C, Lengelle J, Louisanna E, Barantal S, Murat C, Buée M (2017) Diversity and structure of fungal communities in neotropical rainforest soils: The effect of host recurrence. Microbial Ecology 73(2): 310–320. https://doi.org/10.1007/s00248-016-0839-0

Senanayake IC, Maharachchikumbura SSN, Hyde KD, Jayarama Bhat D, Gareth Jones EB, McKenzie EHC, Dai DQ, Daranagama DA, Dayarathne MC, Goonasekara ID, Konta S, Li WJ, Shang QJ, Stadler M, Wijayawardene NN, Xiao YP, Norphanphoun C, Li Q, Liu XZ, Bahkali AH, Kang JC, Wang Y, Wen TC, Wendt L, Xu JC, Camporesi E (2015) Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). Fungal Diversity 73(1): 73–144. https://doi.org/10.1007/s13225-015-0340-y

Steyaert RL (1949) Contribution à l'étude monographique de *Pestalotia* de Not. et *Monochaetia* Sacc. (*Truncatella* gen. nov. et *Pestalotiopsis* gen. nov.). Bulletin du Jardin botanique de l'État à Bruxelles 19(3): 285–354. https://doi.org/10.2307/3666710

Steyaert RL (1953) New and old species of *Pestalotiopsis*. Transactions of the British Mycological Society 36(2): 81–89. https://doi.org/10.1016/S0007-1536(53)80052-5

Steyaert RL (1963) Complementary informations concerning *Pestalotiopsis guepini* (Desmazieres) Steyaert and designation of its lectotype. Bulletin du Jardin botanique de l'État à Bruxelles 33(3): 369–373. https://doi.org/10.2307/3667200

Sutton BC (1980) The coelomycetes: fungi imperfecti with pycnidia aceruli and stromata. Commonwealth Mycological Institute, Kew, Surrey, UK. https://doi.org/10.1016/S0007-1536(81)80170-2

Wang Y, Ran SF, Maharachchikumbura SSN, Al-Sadi AM, Hyde KD, Wang HL, Wang T, Wang YX (2017) A novel *Pestalotiopsis* species isolated from bulbophyllum thouars in Guangxi Province, China. Phytotaxa 306(1): 96–100. https://doi.org/10.11646/phytotaxa.306.1.9

Wang YC, Xiong F, Lu QH, Hao XY, Zheng MX, Wang L, Li NN, Ding CQ, Wang XC, Yang YJ (2019) Diversity of *Pestalotiopsis*-like species causing gray blight disease of

tea plants (*Camellia sinensis*) in China, including two novel *Pestalotiopsis* species, and analysis of their pathogenicity. Plant Disease 103(10): 2548–2558. https://doi.org/10.1094/PDIS-02-19-0264-RE

Wang YF, Tsui KM, Chen SM, You CJ (2024) Diversity, pathogenicity and two new species of *Pestalotioid* fungi (Amphisphaeriales) associated with Chinese Yew in Guangxi, China. MycoKeys 102: 201–224. https://doi.org/10.3897/mycokeys.102.113696

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wu F, Dai SJ, Vlasák J, Spirin V, Dai YC (2019) Phylogeny and global diversity of *Porodaedalea*, a genus of gymnosperm pathogens in the Hymenochaetales. Mycologia 111(1): 40–53. https://doi.org/10.1080/00275514.2018.1526618

Xie J, Li J, Yang YH, Chen YH, Zhao PJ (2014) Two new ambuic acid analogs from *Pestalotiopsis* sp. cr013. Phytochemistry Letters 10: 291–294. https://doi.org/10.1016/j.phytol.2014.10.002

Zhang D, Gao F, Jakovli'c I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Zhao HJ, Shu YX, Doilom M, Zeng XY, Zhang H, Dong W (2024) *Pestalotiopsis phyllostachydis* sp. nov. from Guangdong, China. Phytotaxa 633(1): 68–85. https://doi.org/10.11646/phytotaxa.633.1.8

Supplementary material 1

The concatenated ITS, tef1-α and tub2 sequences

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Data type: fas

Link: https://doi.org/10.3897/mycokeys.109.131000.suppl1